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MANGANESE TOXICITY IN FIELD AND MARKET GARDEN GROPS*

by MARIE P. LÖHNIS

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1. INTRODUCTION

The question of manganese toxicity of the soil as a practical problem in agriculture and horticulture was encountered quite accidentally.

In a field of sandy soil of light texture adjacent to the Laboratorium voor Microbiologie at Wageningen in 1922, a series of 40 permanent experimental plots of 42 m² were laid out. They are well separated by sunken cement plates. Since 1922 these plots have received various fertilizer treatments; each plot received a similar manurial treatment each year. Each plot has always been grown with the same

*) Since 1948 this investigation has been carried out under the National Council for Agricultural Research T.N.O. (Applied Scientific Research).

crops, which however, have been varied yearly. The plots which will be mentioned in the following account are a triplicate series of six, supplied with various amounts of limestone (0-5-10-15-30-60 kg) termed as I_0, I_5—I_{60}, II_0, II_5—II_{60}, III_0, III_5—III_{60} and two plots in duplicate which either have received chilean nitrate (Ch) or ammoniumsulphate (AS) as a nitrogen fertilizer. Under war conditions, however, when chilean nitrate was unavailable, ammoniumsulphate had to be substituted for it. These various treatments have of course greatly influenced the pH of the plots. In Table I the data are

TABLE I

pH of experimental plots												
Series of limed plots	I				II				III			
Date	1946 24/7	1947 20/5	1948 29/5	1949 6/5	1946 24/7	1947 20/5	1948 29/5	1949 6/5	1946 24/7	1947 20/5	1948 29/5	1949 6/5
60 kg l.	6.65	5.15	5.6	6.3	6.57	5.15	5.8	6.2	6.43	5.25	5.8	5.8
30 ,,	5.75	5.0	5.2	6.1	5.85	4.95	5.7	5.95	5.85	4.9	5.3	5.1
15 ,,	5.15	4.5	4.95	5.0	5.15	4.42	4.95	5.7	5.40	4.9	5.1	4.9
10 ,,	4.92	4.32	4.45	5	5.00	4.3	4.95	5.0	5.75	4.95	5.05	5.05
5 ,,	4.95	4.0	4.7	5.1	4.8	4.55	4.85	5.4	5.00	4.45	4.75	4.6
0 ,,	4.42	3.8	4.15	4.8	5.1	4.45	4.8	5.1	4.83	4.4	4.75	4.55
Ch. 1	5.97	4.5	5.55	5.8								
Ch. 2	6.65	4.9	5.15	5								
AS 1	4.57	4.05	3.6	5.15								
AS 2	4.55	4.1	4.85	4.3								

recorded. Since the differences between the limed plots tended to decrease in spring 1949 a fresh addition of limestone in appropriate proportion was made to each of them. In May 1940 war conditions prevailed in the Netherlands and the raising of nutritional crops was the immediate need. Half of each plot was sown with *Phaseolus vulgaris* (Var. b r u i n e b o o n), the seeds of which provide a food rich in protein, and the others halves of the plots were kept in potatoes. Up till 1945 both these crops were grown alternately on the half plots.

Early in the summer of 1940 in the more acid plots an injury to the foliage of the young bean plants was noted. In the leaves a marked marginal chlorosis extending into the intervenal areas occurred (Fig. 1). When older, the leaves became somewhat crinkled and were spotted with small yellowish and later whitish areas (Fig. 2 en 3).

Finally minute brown necrotic spots appeared (Fig. 4) and the petioles of the seed leaves and of the first trifoliate leaves were speckled with small superficial purple-brownish spots. Severely injured plants remained stunted and produced hardly any flower or seed. Less injured plants were often seen to recover later in the season, when only traces of the initial injury still occurred in the older leaves. Owing to the injury in the young stage, the final yield was reduced. In all successive years the same symptoms could be noted.

The literature then available did not furnish any description of this injury, so its cause was quite unknown.

2. THE CAUSE OF THE INJURY IN BEANS (*Phaseolus Vulgaris*)

a. Experimental induction.

In 1943 some light was thrown on the cause of the damage. In a variety of bean (van Tol's bruine boon) on certain soils the occurrence of an internal necrosis in the seeds had been noted; a phenomenon very much like "marsh spots" in peas, caused by a deficiency of manganese. In order to establish, whether this injury could be ascribed to a similar cause, solution cultures deficient in manganese were set up. At the same time a few vessels received a large supply of $MnSO_4$ (100 and 50 mg/l) in order to demonstrate the symptoms of manganese excess in beans. Quite unexpectedly the symptoms induced in the latter cultures appeared to be strikingly similar to those already noted in the field.

In the meantime the same injury had been observed in agricultural fields on sandy soil in the neighbourhood of Wageningen. Under normal conditions such fairly acid fields would not have been grown with beans, but there also war conditions had favoured the raising of this crop. Frequently the injury could be recognized at a distance by the bright yellow colour of the leaves. It appeared then that the French bean (dubbele prinsesseboon), the pods of which are used as a vegetable, was remarkably susceptible and in later years "dubbele prinsesseboon" has been chiefly grown as the experimental plant.

b. The manganese content of the leaves.

The first means of attack on the problem was the estimation of the amount of manganese in the foliage of healthy and of affected plants. The youngest fullgrown leaves were collected and comparable sam-

ples were analysed according to the method of Marshall[14]) amended by Steenbjerg[24]).

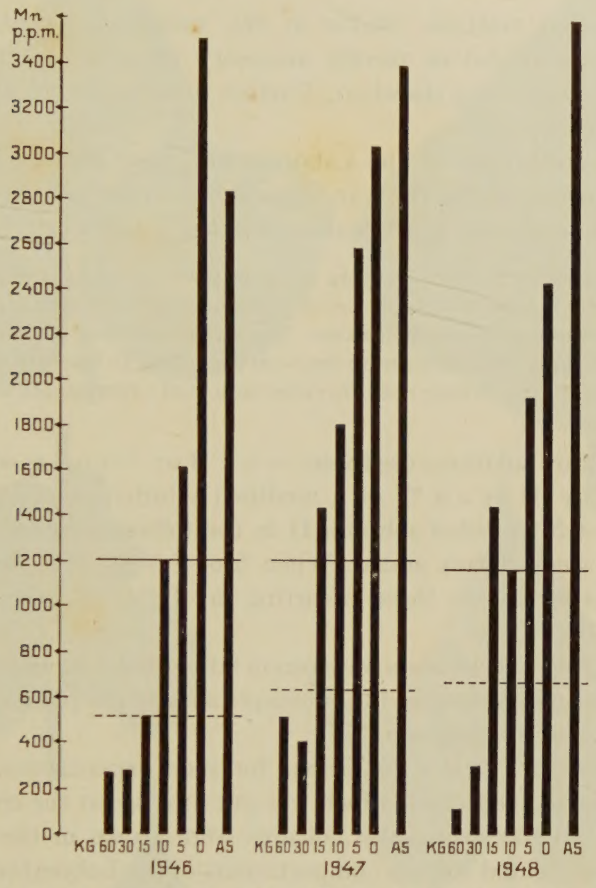
A sample of 0.5–0.05 g dry weight is transferred to a Kjeldahl flask, a mixture of 5 ml of HNO_3 (5p) and 4 ml of H_2SO_4 (6p) are added and the flask is heated at first on a low flame, which is later increased, until the liquid is colourless. After the addition of 15 ml. dist. water the solution is rendered alkaline with 6 N NaOH. Conc. H_2SO_4 is then added until it is just acid and 1 ml is then added in excess. The solution is filtered into a 100 ml flask, 2 ml of 0.1 N $AgNO_3$ are added and the flask filled to the mark with distilled water. After filtering into a beaker, 1 ml of 6 N HNO_3, and a few mg of ammonium persulphate are added to the filtrate which is heated almost to the boil. After cooling the manganese as manganese permanganate is estimated in a Pulfrich photometer.

In the following account the content of manganese is always expressed as ppm of dry matter.

In the samples collected in 1943 a very marked difference in content appeared to exist between young healthy foliage (100—500 ppm) and injured foliage of the same age (1000—3000 ppm). (Löhnis[12]).

In 1944—45 no further progress was possible, but since 1946 samples of bean foliage (Prinsesseboon) have been collected from the 28 plots under investigation. The first samples were always taken as soon as symptoms of injury became conspicuous, generally during the development of the first trifoliate leaf and all plots were sampled simultaneously. In Gr. 1 the results obtained in 1946—48 in series I and AS_1 are presented. For the sake of conciseness the data for the plots, in series II and III and AS_2 which correspond well with those in Series I are not given. Although the amounts recorded in the same plot in various years may vary widely, the trend in the series of limed plots was always similar. The most striking result was, that the threshold values above which the foliage was always visibly affected (marked by a drawnout line in the graphs) was hardly seen to vary in the successive years. These values were in 1946 1210; in 1947 1211; in 1948 1167 and in 1949 1104. The values found in the healthy leaves ranged from 40—904, in injured leaves from 1104—4216. In the AS plots the injury was always the most severe and the corresponding content of manganese very high.

When the initial injury has not been very severe plants are apt to recover and in fully developed plants traces of injury may be noted only in the oldest leaves. Will the upper leaves of such plants contain



Gr. I. Content of manganese in the foliage of bean plants from the plots of Series I and AS_1.

———— Lowest level of affected plants.

– – – – – – Highest level of healthy plants.

amounts of manganese below the level which causes injury in young plants? This frequently appeared not to be the case. In the tops of these healthy plants values might be found exceeding those recorded in young injured plants. These higher values ranged between 1296 and 1681. Hence more mature plants appear to tolerate higher amounts of manganese.

c. Solution cultures.

A close correlation between manganese content and the occurrence of injury does not, however, furnish strict proof for an

actual causal relation. Shifts in the manganese content may easily be conceived as merely accessory phenomena, the actual cause, however, lying elsewhere. Further proof by means of solution culture was needed.

As the greenhouse of the Laboratorium voor Microbiologie had been destroyed during the war, experiments could only be run on a small scale and were dependent on weather conditions.

Glass vessels of 1 l capacity were used wrapped in corrugated cardboard. The lids were moulded from plaster of Paris and provided with holes.

4 plants were grown in each vessel. Tap water was used for the solutions and added daily. Solutions were renewed according to the rate of development of the plants. Where not otherwise indicated experiments were ru in duplicate.

The culture solutions used were von der Crone solution as modified by Bryan*) or a modified solution of Zinzadze (pH 5.5—6.5 †)) called solution II in the following account. Minor elements were always added. When 5 or 7.5 mg Mn/l was added symptoms similar to those occurring in the field were induced. (Fig. 5—7).

When these experiments were run in late summer however, results were sometimes erratic and the susceptibility of the plants appeared to be less than in early summer.

1949 provided better conditions for such experiments. A small warehouse had been built which was put into use at the end of May and early in the spring also cultures were grown in the properly heated and lighted culture compartments of the Laboratorium voor Plantenphysiologisch Onderzoek. Culturing under these conditions offered an explanation of difficulties met with in former years.

On Febr. 27th cultures in von der Crone solution provided with 0.5, 5 and 10 mg Mn/l had been set up. By March 8th none of these plants had shown any signs of injury, although the doses of 5 and 10 mg were high enough to warrant the expectation of the occurrence of signs of damage. On March 9th the temperature in the afternoon rose so high that it was feared that the plants might wilt so they were moved to a more moderately heated compartment of

*) Von der Crone solution: KNO_3 1 g; $CaSO_4$ 0.2 g; $MgSO_4.7H_2O$ 0.2 g; $Ca_3(PO_4)_2$ 0. 2 g; $FePO_4$ 0.2 g; per l. tapwater. Minor elements added to all solution cultures: H_3BO_3 0.5 mg; $CuSO_4$ 0.125 mg; $ZnSO_4.7H_2O$ 0.5 mg.

†) Solution II: KNO_3 0.5 g; NH_4NO_3 0.2 g; KCl 0.36 g; $Ca_3(PO_4)_2$ 0.5 g; $MgSO_4.7H_2O$ 0.25 g; $Fe(NH_4)_2(SO_4)_4.24H_2O$ 0.175 g.

3

2

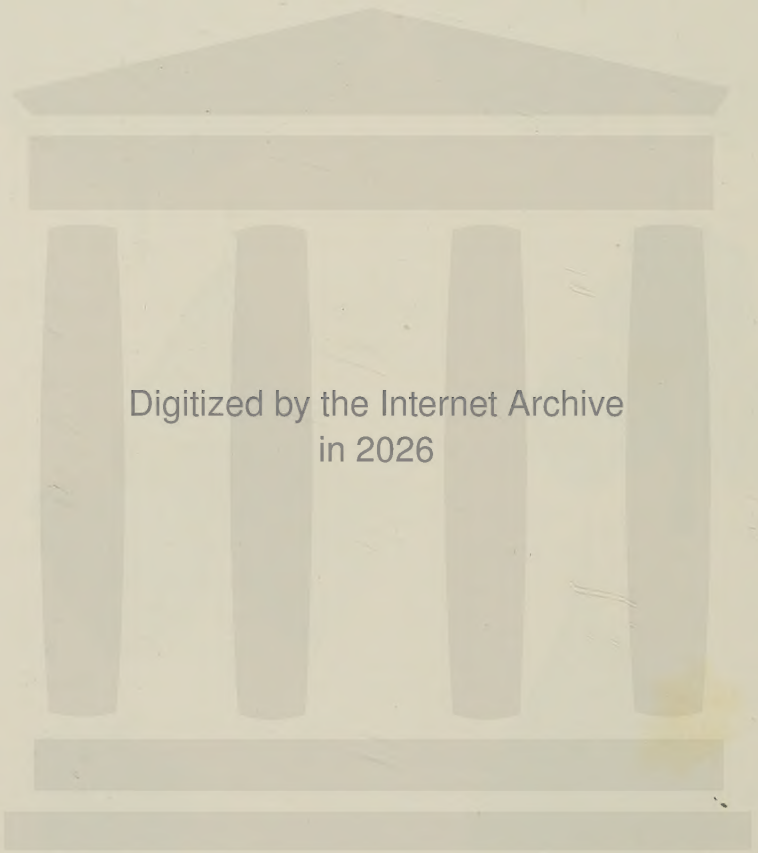
4

6

5

7

2. Bean (Prinsesseboon), Unlimed plot. — 3. Bean (Prinsesseboon), Unlimed plot.
4. Bean (Prinsesseboon), Unlimed plot. — 5. Bean (Prinsesseboon), 10 mg Mn/l.
6. Bean (Prinsesseboon), 7.5 mg Mn/l. — 7. Bean (Prinsesseboon), 7.5 mg Mn/l.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the greenhouse. On March 10th all those plants which had been supplied with 5 and 10 mg Mn l showed the characteristic injury all over their foliage. Hence at the former higher temperature manganese had apparently been taken up by the plants without the induction of any visible signs of injury.

On March 21st a further comparative test was made with plants in von der Crone solution, provided with 10 mg Mn/l. 3 vessels were put in a warm compartment and 3 in a more moderately heated one. On April 8th all plants in the latter compartment were markedly injured. In the hot compartment plants in two of the vessels were quite healthy, but in the 3rd vessel the plants were injured. The foliage of all these plants was harvested and the content of manganese estimated. The following amounts were found:

at moderate temperature	injured	2938 ppm
„ high „	injured	2635 ppm
„ „ „	healthy	2635 ppm
„ „ „	healthy	1496 ppm.

Hence at high temperature 8 plants had withstood without any visible injury, a content of manganese which under the usual temperature conditions of spring would have given rise to severe damage.

From May 2nd—25th beans were again grown in von der Crone solution in the presence of 2.5, 5 and 7.5 mg Mn/l in the heated compartment. None of these showed any signs of injury although the content of manganese in plants grown in the presence of 7.5 mg Mn was estimated as 1780 ppm. From June 22th to July 17th the behaviour of bean plants in von der Crone solution supplied with 0.5, 7.5 and 10 mg $MnSO_4$ was compared when cultivated either in the well ventilated warehouse or in the heated compartment.

The photograph (Fig. 8) of plants supplied with 7.5 mg/l shows the very marked difference. This experiment will be referred to later.

Although the exact conditions of temperature have not been studied it does appear that temperature may act strongly on the occurrence of visible injury, so that comparative investigations of susceptibility must be run under controlled conditions. This influence of temperature may account for the conflicting results I obtained in former years during periods of great heat in summer.

Other external conditions may also play a part. An influence of shading on the occurrence of manganese injury has been recorded by Mc Cool [15]). In greenhouse experiments a heavy shading prevented the occurrence of

any visible injury in beans and tobacco. In beans, however, the shaded plants had a markedly lower content of manganese (1330 ppm) than the unshaded (2270 ppm). Hopkins *et al.* [10]) suggested that a greater exposure to light could bring about particular symptoms of manganese toxicity in pineapples. Shaded leaves, however, without any visible symptom had high contents of manganese similar to the affected ones in the light.

When plants are grown in solutions varying in composition the susceptibility to excess of manganese may vary greatly. This had been noted in former years, but the need of comparing the degree of susceptibility under identical external conditions has only recently been established, and the evidence gathered is somewhat scanty.

In solution Zinzadze pH5 *) supplied with 10 mg Mn/l in 3 vessels kept at moderate temperature quite healthy plants were produced, and only the seed leaves showed the minute, superficial purplish speckling which indicates an excess of manganese. Seed leaves were harvested separately from the remaining foliage; the former contained 1318 ppm Mn, the latter 734. The pH in this solution was measured as 4.8 and when checked with the initial culture solution all manganese was proved to be in solution.

A further instance of the influence of the composition of the culture medium is furnished by the following experiments, run from March 21th till April 8th. Two lots in triplicate, were set up, one with von der Crone solution, the other with the Zinzadze solution pH5, all supplied with 10 mg Mn/l. Whilst in the former solution all plants were markedly injured, the plants in the latter had remained healthy. In von der Crone solution the foliage contained 2938 ppm, in Zinzadze solution 591. In the latter medium all manganese when checked at the start proved to be in solution, whilst in the von der Crone medium only 6.3 mg Mn/l was in solution.

The difference in plant behaviour cannot be explained by a difference of solubility of manganese in the culture medium. The part played by antagonistic action of constituents of the culture solution will be discussed later.

d. Soil cultures.

The experiment, however, which furnishes the most convin-

*) NH_4NO_3 0.334 g; KNO_3 0.166 g; $Ca_3(PO_4)_2$ 0.7 g; KCl 0.616 g; $MgSO_4.7H_2O$ 0.5 g; $CaSO_4$ 0.5 g; $Fe_2(SO_4)_3$ 0.25 g. 1 l tapwater. Minor elements as usual.

Fig. 1.

Fig. 8.

Fig. 9.

Fig. 11.

Fig. 1. Bean plant (Bruine boon) in an unlimed plot
(Photo Plantenziektenkundige Dienst Wageningen).

Fig. 8. Bean (Prinsesseboon), v. d. Crone solution, 7.5 mg Mn/l
Left moderate, and right high temperature.

Fig. 9. Bean (Prinsesseboon), 1. unlimed soil, 2. $CaCO_3$,
3. $CaCO_3 + MnSO_4$.

Fig. 11. *Vicia sativa*, unlimed plot.
(Photo Plantenziektenkundige Dienst Wageningen).

Fig. 12. *Vicia sativa*,
Unlimed plot and 60kg lime.

Fig. 15. Lucerne,
Solution II,
0.5 mg Mn/l and 5 mg Mn/l.
(Photo J. Boekhart)

Fig. 18. Red clover,
v. d. Crone solution,
10 mg Mn/l and 0.5 mg Mn/l.

Fig. 16. Red clover,
Unlimed plot and 60kg lime.

Fig. 17. Red clover,
Unlimed plot.

cing proof that manganese excess is the actual cause of the injury which occurs in the field is the following (L ö h n i s [13]). Bean plants were grown in glass dishes (N e u b a u e r dishes) provided with 400 g soil taken from plot I_0, giving rise to an affected crop (1). The soil in part of the dishes was mixed with 0.8 g $CaCO_3$ (2) whilst to half of the number of the latter 0.25 g $MnSO_4$ was also supplied (3). In the untreated soil (1) young bean plants showed symptoms similar to those noted in the field, in (2) normal healthy plants developed, whilst in (3) symptoms developed exactly similar to those in (1) (Fig. 9).

Here acidity as such has been eliminated as a possible causal agent and the injury is doubtless due to the manganese sulphate added.

This test may easily be carried out with large seeds. When seeds are small, however, it takes a longer interval of time before the plants have developed sufficiently to show symptoms of injury. In the meantime under more alkaline conditions the Mn^{++} may have been oxidised and as insoluble MnO_2 will no longer be available to plants.

3. SUSCEPTIBILITY OF OTHER CROPS

a. Literature.

The question of manganese toxicity has been studied in widely separated regions. The fact, that most of these investigations have been carried out simultaneously with the present one and that foreign literature has been unavailable during a long period, may explain that the facts reported in literature came under my notice for the most part a long time after their publication.

P a r b e r r y [20]) reports on "scald" in beans occurring on certain soils in New South Wales. The affected plants have a higher content of manganese, more especially those which are deficient in magnesium. As the symptoms are not described in detail, it is not certain that this phenomenon is identical with the above.

W a l l a c e, H e w i t t and N i c h o l a s [25]) and H e w i t t [6a]) induced the symptoms of manganese toxicity in runner beans and cauliflower by means of sand solution cultures. The symptoms described in beans correspond closely with those noted at Wageningen and personal discussion in 1947 corroborated this more fully.

H e w i t t [7] [8]), aiming at a resolution of the factors in soil acidity by means of sand solution cultures has studied very thoroughly the effect of manganese excess upon a number of farm and market garden crops.

The description of the symptoms, where the same crops have been studied generally agrees closely with those I have noted.

Tobacco has been reported by Jacobson and Swanback[11]) to be susceptible to manganese excess which occurs in soils in Connecticut. Amounts recorded in damaged plants ranged between 5250–11670 ppm. Bortner[2]) reported the occurrence of similar injury in Kentucky.

Fried and Peech[5]) report that liming of the soil reduced the Mn content of the leaves of lucerne to $\frac{1}{8}$ of that found in the control. Added manganese proved distinctly toxic.

Peech and Bradfield[21]) point to the part manganese and aluminum may play in explaining the behaviour of lime-responsive crops.

Morris and Pierre[19]) have studied the minimum concentration of manganese which is necessary for injury in various Leguminosae in a given nutrient solution. Average yields and characteristic symptoms are described. The plants differed greatly in the amounts of manganese absorbed. At least two factors affect the tolerance of the plant, viz. the amount of manganese absorbed and the relative ability to endure large amounts within the plant.

C. R. Millikan[16]) describes in excess manganese cultures a characteristic type of leaf necrosis in flax (at concentrations in the solution of 25 ppm or more). Identical symptoms were also produced in highly acid soils by heavy applications of manganese sulphate. With excess manganese (25–150 ppm), the addition of molybdenum (5, 10 and 15 ppm) retarded the date of appearance and reduced the severity of lower leaf necrosis. This effect of Mo was confirmed in acid soils with heavy dressings of manganese sulphate.

In 1948[17]) he reported on an antagonism between manganese and molybdenum for peas, cabbage and tomatoes in solution cultures.

Sherman and Fujimoto[21a]) describe a disease of lettuce in Hawaï, termed "yellow leaf fringe", which could be controlled by treatment of the soil with mulch or lime. The manganese content of plants from untreated soil was 3800 ppm while in healthy plants it did not exceed 760.

Du Plessis[4]) suggests manganese excess as the cause of an injury of unknown origin in vine leaves.

Daji[3]) Manganese toxicity is suggested as a probable cause of the band disease of Areca palm.

b. Experimental results.

Neither potatoes (var. Eigenheimer) grown side by side with the beans nor strawberries, both crops grown for other purposes on these plots, had ever shown any signs of injury. Starting in 1947 various crops were grown in order to ascertain their comparative susceptibility. These crops were chosen from among those known to be limeresponsive or from those which were recorded as unsusceptible to manganese deficiency (grey-speck disease) such as *Vicia sativa*.

10. *Vicia sativa*, Unlimed plot. — 13. *Vicia sativa*, Soil culture, $CaCO_3$ and $MnSO_4$. — 14. Lucerne, Unlimed plot.

Vicia sativa.

Field test. When grown in 1947 vetch showed very striking differences in development in the various plots. In plots I_0 and $AS_{1 \text{ and } 2}$ it was a complete failure. In the highly limed plots the plants developed profusely. A definite pattern of injury of the leaves occurred, viz. a chlorosis of the young leaves and a very marked dark purplish discoloration along the margins of the fuilgrown leaves. Sometimes small more orangered sunken spots occur in the leaf margins and the upper surface of the leaves may be speckled with minute dark spots. The whole plant remained small and spindly (Fig. 10, 11 and 12).

Purple margins may also be induced by potassium deficiency. Whilst excess manganese, however, induces this injury in the leaves of very young plants, the symptoms of potassium deficiency occur in more mature plants and on the lower leaves. These symptoms could be noted sometimes in large plants on the highly limed plots.

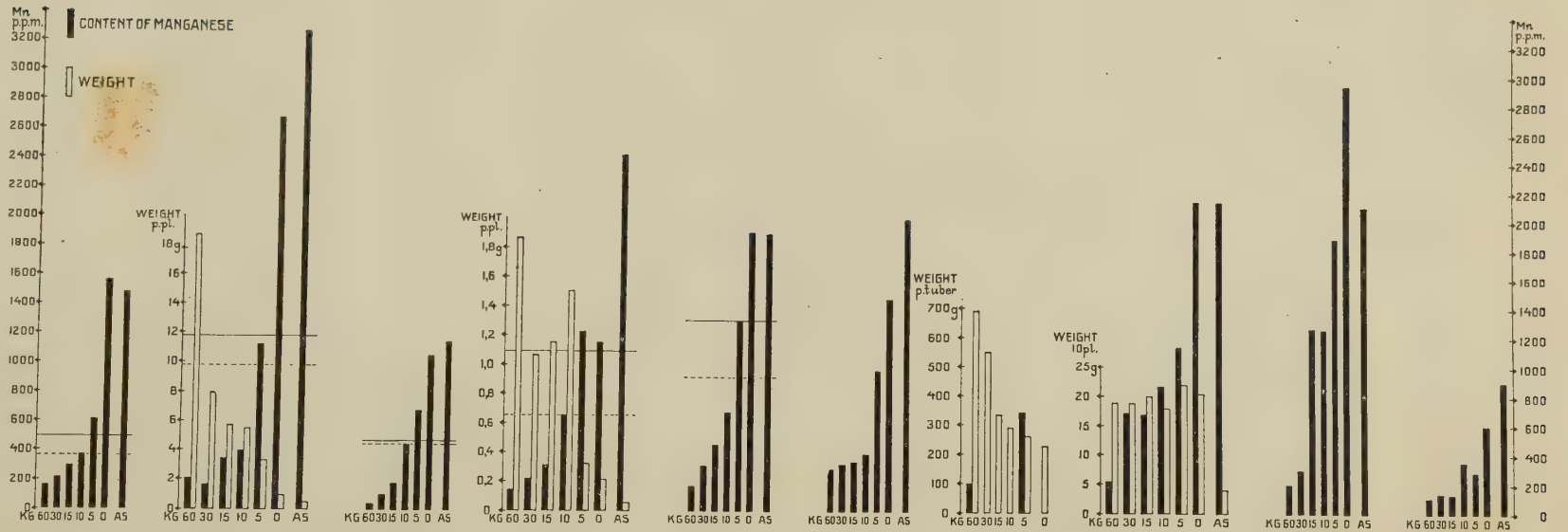
In 1948 June 11th young leaves were sampled in all plots. Gr. II gives the data for the manganese contents in Series I and AS_1. In 1949 on June 17th plants were cut at soil level (4 in each plot) and weighed. Fresh weight per plant and manganese content are given in Gr. II. A marked negative correlation can be noted. The minimum content of affected plants was 1117.

Solution cultures. The symptoms caused by manganese excess were exactly reproduced in solution cultures. In von der Crone solution a dose as low as 2.5 mg Mn/l induced slight injury. The leaves of such plants contained 1021 ppm. 5 mg Mn/l induced marked injury and in the leaves a content of 1524 ppm was estimated.

Soil cultures also such as described on p. 201 furnished very clear-cut results. The injury in plants in the untreated soil agreed very closely with that in the soil treated with $CaCO_3$ and $MnSO_4$ (Fig. 13).

Medicago sativa.

Field test. Lucerne, known as a lime-responsive crop was grown in 1948 and 1949. A very marked delay in development in the more acid plots was noted. In this stage definite signs of injury occur in the leaves: for instance the leaflets are brightly yellow often with brown necrotic spots, and a narrow yellow margin extends towards the base. In older leaflets a browning and



Vicia sativa 1948 | *Vicia sativa* 1949 | *Lucerne* 1948 | *Lucerne* 1949 | *Trifolium pratense* 1949 | *Trifolium repens* 1949 | *Mangold* 1949 | *Flax* 1949 | *Tobacco* 1947 | *Mustard* 1948

Gr. II. Content of manganese in the foliage of various crop plants from the plots of Series I and AS_1.

———— Lowest level of affected plants. – – – – – – – – Highest level of healthy plants.

scorching of the margins occur (Fig. 14). Sometimes sunken discoloured spots occur in the marginal zone of the leaflets, reminiscent of symptoms of potassium deficiency. But here again these spots occur on the younger leaves of young plants. Many plants die off. Those that can stand the initial damage catch up with healthy plants so that when mature they show hardly any signs of injury. The symptoms described by Hewitt [8]) correspond closely with these.

The manganese of young leaves was estimated in 1948 and 1949. On June 20th 1949 representative samples of young plants were collected and weighed. In Gr. II the data are presented; a high content of manganese is correlated with low weight. The minimum content of manganese in affected plants was at a much higher level (1083) in 1949 than in 1948 (477).

Solution cultures. Here again it was possible to reproduce the field symptoms. 2.5 mg Mn l in von der Crone solution induced slight injury, in solution II the marginal discoloration occurred in the first leaves only. When 5 mg Mn/l was supplied the leaves of the plants in both solutions were much smaller and very chlorotic (Fig. 15). The content of manganese of these plants was estimated and corresponded with the degree of visible injury.

Culture solution	mg/Mn/l	Mn ppm
von der Crone	2.5	1022
,,	5	2243
Solution II	2.5	588
,,	5	2115

Trifolium pratense.

Field test. Red clover was only grown in 1949. As the spring was exceptionally dry, the germination was very irregular and a valid comparison of the development in the various plots could not be made. Young plants (June 10th) in the acid plots were small (Fig. 16) and the leaflets showed narrow greenish chlorotic margins. In course of time the plants recovered in all but the AS plots which bore hardly any plants. In the most acid plots the outer (oldest) leaves of the mature plants sometimes still showed chlorotic margins with a dark necrotic outer edge, and indeed the chlorotic areas might extend up to the main rib and the leaf area might be crinkled (Fig. 17). Such plants, however, flowered normally and quite normal younger leaves developed.

The symptoms correspond with those described by Hewitt [7]).

Samples were taken at a time when the differences in development were still conspicuous. In Gr. II the very marked influence of the degree of liming on the content of manganese can be seen.

Solution cultures. In von der Crone solution supplied with 5 mg Mn/l set up on May 23rd plants showed on June 8th small necrotic light green spots in the marginal zone. These plants were somewhat smaller than the controls. Later these plants became quite healthy. Plants supplied with 10 mg Mn/l were quite small on June 8th and the leaves bore many chlorotic and necrotic spots. Fig. 18 taken June 22nd shows them still lagging far behind the controls in development, although the younger leaves were healthy and of the same size as those of the controls. On July 9th they had caught up with the latter.

In Solution II the same amounts of $MnSo_4$ affected the plants in a lesser degree. A second series of solution II, set up in triplicate (July 12th—Sept. 9th) with amounts of 0.5, 10 and 15 mg Mn/l, showed on August 5th chlorotic margins in the presence of either 10 and 15 mg Mn/l. On September 9th the development in the 10 mg Mn and in two of the 15 mg Mn vessels was profuse, but in one of the latter it was weak. Controls and plants in a 10 mg Mn vessel had been infested by grasshoppers. The following contents of manganese were recorded:

mg Mn/l	number of plants	fresh weight per plant g	Mn ppm
10	4	9.5	1318
10	4	9.1	1253
15	3	11	1631
15	3	11.3	
15	3	3.7	2208

Hence more mature plants can stand a high content of manganese without damage. Both field trials and solution cultures show that older plants are markedly more tolerant for manganese than young plants.

Trifolium repens.

Field test. White clover was only grown in 1949 and here again weather conditions acted very unfavorably on germination. Young plants in the acid plots lagged greatly in development; actual symptoms of injury, however, were not recorded. Later these plants caught up with the others and finally plants in the

acid plots could not be distinguished from the initially healthy ones. Samples taken on August 8th in the field show the influence of the liming on the manganese content (Gr. II).

Solutions Cultures. In von der Crone solution (July 15th—August 16th) very young plants did show in the presence of 2.5 and 5 mg Mn l some minute necrotic spots, but at an early date these could no longer be seen. Plants supplied with 5 mg Mn/l, when young, produced small leaves and lagged in development. Fig. 19 taken on August 16th, however, shows that by then the latter plants had started to grow better, the young leaves were then of a same size as those of the control plants. The content of manganese in ppm, in the foliage of these plants was 813 as against 164 in the controls.

In an experiment in solution II (August 12th—October 4th) the final difference between cultures supplied with 5 and 0.5 mg Mn/l was very slight; in the presence of 10 mg Mn/l foliage and roots were smaller (Fig. 20). The content of manganese in the foliage of these plants was 160 (0.5 mg), 726 (5 mg) and 1140 (10 mg) ppm.

Mangold.

Field test. Mangolds (var. Groenkraag) were grown in 1949. On June 29th the young plants in the acid plots were markedly depressed in growth (Fig. 21). During further development no visual signs of injury were noted, the depression of growth was, however, maintained. In the AS plots the crop failed almost completely. When in the early days of November roots were dug their weight corresponded with the foliar development. In Gr II the weight of the roots and the manganese content of the foliage, as far as this has been estimated, is recorded for series I and AS_1.

The contents of Mn increase inversely with the degree of liming, but do not nearly reach the high values recorded in the other crops. The maximum found in all the plots was 947. Although doubtless some adverse action is exercised in the more acid plots, it remains to be proved that excess of manganese is the actual cause.

Hewitt[7]) reports that sugar beet is remarkably tolerant of high concentrations of manganese in spite of its liability to fail on moderately acid soils.

Solution cultures. Plants in von der Crone solution in the presence of 5 mg Mn/l, which induced a content of 1424 ppm in the foliage, actually exceeded in fresh weight of the foliage

(84 gr 12 pl.) the controls the fresh weight of which was 77 g (12 pl.), whilst containing 249 Mm ppm. In the presence of 10 mg Mn/l a slight depression in growth could be observed, viz. 59 g (in 10 plants, two plants having succombed in the course of the experiments). These ten plants contained 1650 ppm. As this growth depression together with a high content of manganese was so much slighter than the depression noted in the field along with a much lower content, the latter could not presumably be ascribed to manganese toxicity. This experiment will be referred to in a later paragraph. These cultures showed in addition an unfamiliar symptom. In the presence of 5 and 10 mg Mn/l some of the leaves were of an uniform yellowish or nearly white shade, whilst after the supply of 15 mg Mn/l all of the leaves were narrow and white. When areas of such discolored leaves were painted over with $\frac{1}{4}\%$ $FeSO_4$ solution they became a bright green after two days. Thus an iron deficiency was confirmed. This phenomenon will be discussed later.

H e w i t t [7]) mentions that mangold cultures, which have been provided with a large amount of manganese, produced young leaves abnormally narrow, with paling margins, followed by a yellow chlorosis, probably due to iron deficiency.

In solution II 10 and 15 mg Mn/l produced no such symptoms, only a slight depression in growth.

F l a x.

F i e l d t e s t. Flax (Var. C o n c u r r e n t) grown in 1949 showed no difference in development or any sign of injury in any of the unlimed plots. Only in the AS plots the plants were very small and the lower leaves yellow with dry brown tips (Fig. 22). On June 17th 10 representative stems cut at the level of the cotyledons were gathered from each plot and the fresh weight determined. In Gr. II the weights of plants and the manganese content of the leaves are recorded. Whilst it shows clearly that the increase in manganese content runs the familiar course, reaching maximally 2137 ppm, no correlation with the yield of young plants could be ascertained. Only in the AS plots could a very marked growth depression along with a high content of manganese (2134 ppm) be noted. Hence field experiments point to a marked tolerance.

H e w i t t [7]) too did not observe any action of manganese excess. This is however not in accordance with data furnished by M i l l i k a n [16]), who claims to have induced a "lower leaf scorch" by excess

Fig. 19. *Trifolium repens*,
v. d. Crone solution,
5 mg Mn/l and 0.5 mg Mn/l.

Fig. 20. *Trifolium repens*,
Solution II,
10 mg Mn/l, 5 mg Mn/l and 0.5 mg Mn/l.

Fig. 21. Mangold,
Unlimed plot and 60 kg lime.

Fig. 22. Flax,
AS plot and 60 kg lime.

Fig. 24. Bean (Prinsesseboon),
7.5 mg Mn/l, showing iron deficiency, and 0.5 mg Mn/

Fig. 23. Flax,
v. d. Crone solution (I) and solution II
5 mg Mn/l.

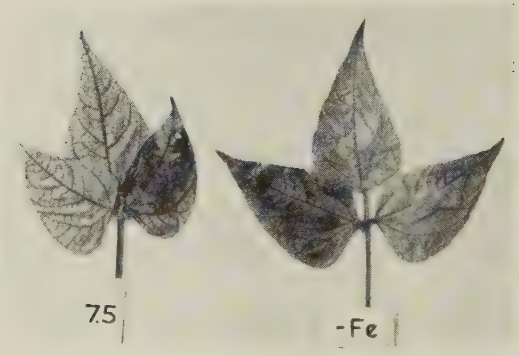


Fig. 25. Bean (Prinsesseboon),
7.5 mg Mn/l and iron omitted,
both treated with $FeSO_4$.

of manganese, which could be reduced by application of molybdenum. It has not been ascertained, whether the field symptoms observed in AS plots, and which might be described as "lower-leaf scorch" do respond to a supply of molybdenum.

Solution cultures. In von der Crone solution with 0.5 mg Mn l very healthy plants were grown. A supply with 0.5 or 1.0 mg sodium molybdate did not influence the development. In the presence of 5 and 10 mg Mn l with or without molybdenum the plants remainded very short and the foliage was a very pale green or quite white. Painting these leaves with either $\frac{1}{4}\%$ $FeSO_4$ or 0.0025% $Na_2MoO_4.2H_2O$ was ineffective. The contents of the leaves were 1211 (5 mg Mn l) and 1261 (10 mg Mn/l). These symptoms did not correspond with those noted in AS plots or those described by Millikan as "lower-leaf scorch".

In solution II with 5 mg Mn l the largest plants grew (Fig. 23). The manganese content of these plants was 940 ppm. Even in 10 mg Mn/l one plant reached a height of 17 cm (content 1232 ppm), but 4 were much smaller and strongly chlorotic (content 1816 ppm). Here again was a supply of molybdenum ineffective.

Since these results with flax are conflicting, the behaviour in solution cultures needs further investigation.

Potatoes.

Field test. In potatoes (Var. Rode Eigenheimer) in none of the plots could any injury be noted. In 1946 young leaves were sampled in some of the plots. The highest values recorded were in Plot II_0 1620, III_0 1717, AS_1 2225, AS_2 1771.

Potatoes which are known to thrive well on moderately acid soils appear to tolerate fairly high contents of manganese without injury. Hewitt[7]), however, describes a characteristic injury induced by a moderate level of manganese, stem specklings or lesions being the most frequent symptoms. It may be conceived that the tolerance might vary in different varieties and further investigation is planned.

Tobacco.

Field test. When grown in 1947 no symptoms of injury could be noted. A close correlation, however, could be established between the manganese content of the young leaves and the amount of liming of the plot (Gr. II). High contents of manganese were recorded (max. 2936 ppm), so that tobacco appeared to be highly tolerant. The amounts recorded by Jacobson and

Swanback in affected plants, which varied between 5250 and 11670 ppm, greatly exceed the amounts recorded here.

Mustard.

Field test. In 1948 mustard was grown. In none of the plots did any difference in growth occur and all plants were unaffected. Samples of young leaves were taken on June 9th before flowering began. In Gr. II the contents of manganese are recorded. Although the amounts of manganese increase along with a decrease in liming (max. 598 ppm, in AS 901) all amounts remain very much below the level reached by other crops. This insensitiveness of mustard is doubtless due to a low intake of manganese. The behaviour of mustard in solution cultures and the level tolerated therein has not been tested. Hewitt [7]) reports the occurrence of symptoms of injury in mustard.

Oats.

In acid plots magnesium may be washed out from the soil so causing disorder in crop plants. As oats are most susceptible to magnesium deficiency, they were grown in 1944 in some of the plots in order to establish whether symptoms of magnesium deficiency might occur. In none of the plots were the oats affected. The amounts of manganese recorded in the young leaves were:

kg lime	0	60
Series I	314 ppm	87.2 ppm
,, II	301 ppm	—
,, III	370 ppm	89 ppm

The amounts of manganese absorbed by oats are much below the level of other plants. This may explain their susceptibility to manganese deficiency in more alkaline soils. (Steenbjerg [24]).

Strawberry.

In none of the plots did any of the plants appear affected. Plants seemed to thrive even better in more acid soils. In 1946 some samples were analyzed; the highest amounts found were in the three unlimed plots, viz. 1264, 1641 and 1220 and in an AS plot 1328.

Peas.

Field test. The very dry spring 1949 made the growth of peas in the experimental plots a failure.

Solution cultures. In von der Crone solution

peas throve well in the presence of 10 and 15 mg Mn/l; i.e. amounts that would severely affect other crop plants. All plants were harvested at the budding stage. The amounts of manganese in the foliage were:

in the presence of 10 mg Mn/l 783 and 837
„ „ „ „ 15 „ „ 897 „ 1282.

Peas had apparently taken up little manganese under these conditions and a susceptibility is hardly likely.

The field test is to be repeated.

Broad beans.

Field test. The field crop in 1949 did not yield any clear-cut results as a severe infestation by black aphis resulted in a very irregular stand.

Solution cultures. An experiment in von der Crone solution gave the following results:

mg Mn/l	5		10		15	
Fresh weight g	63	(7 pl.)	65	(7 pl.)	74	(8 pl.)
Mn ppm in yield of vessel	787	641	1489	1766	1645	1795

Plants throve uniformly well in all the vessels and indeed the fresh weight of plants grown in the presence of 15 mg Mn/l was highest. The fact that a content of 1795 ppm did not affect the plants, points to tolerance.

Hewitt [7] [8] considers broad beans unsusceptible.

Lettuce.

Sherman and Fujimoto [21a]) claim that injury of lettuce consisting in a yellowing of the leaves ("yellow leaf fringe") is due to manganese excess. Since in the culture of lettuce an injury known as "tipburn" (Andersen [1]) (in the Netherlands called "randjes") is known, the cause of which has been uncertain up to now, I prepared some solution cultures (1 plant per vessel) in order to get acquainted with such damage as manganese excess might induce. The lettuce throve very well in these culture solutions and headed normally.

In the variety „Wonder van Voorburg" 12.5 mg Mn/l in von der Crone solution induced a content in the foliage of 2023 ppm, in solution II 25 mg Mn/l 1766. The only slight injury that could be noted consisted of a paling of the outer margins along

with a darkening of the veins, more or less reminiscent of bacterial injury. The pattern of "tipburn" injury was not reproduced. Nor was this the case in the variety "New York" in solution II, where a dose of 12.5 mg Mn/l induced a content of 1638 in the foliage and only a slight paling of the margins. Even in the presence of 25 mg Mn/l plants were hardly affected.

Hence it seems unlikely that manganese excess plays a part in the causation of "tipburn".

Hewitt[8]) describes a manganese induced injury in which the foliage is pale and dull yellow around the leaf margin.

Vine.

Du Plessis[4]) suggests that a mottling of vine leaves may be due to manganese excess. This led me to try to determine whether manganese excess might affect the variety "Black Alicante" in the same manner.

Cuttings were made to root and grow in large flower pots filled with soil taken from the plots I_0, I_{60}, AS_1 and Ch_1. The plants were grown from April 14th–Sept. 3rd in a greenhouse. In none of the plants could any injury in the foliage be noted. The leaves were gathered and the following data were recorded.

Soil from plot	Mn ppm in leaves per pot		
I_0	395	482	
I_{60}	273	182	
AS_1	477		
Ch_1	224	214	157

In all the foliage the content of manganese was low, so that any risk of injury due to manganese excess was hardly probable.

In addition, cuttings were grown in von der Crone solution, one cutting per vessel, all having been rooted in the presence of 0.5 mg Mn/l. The amounts recorded in the leaves were:

Mg Mn/l	0.5	12.5	20	50
Mn ppm	222	388	553	342
per cutting		620		865

The foliage of all the cuttings was unaffected and here again the content in the presence of much manganese was low. Hence these

experiments do not point to the probability that the injury is in any way due to manganese excess.

As the cuttings have not been grown into adult vines these data are not considered to furnish a definite answer.

c. Discussion.

When the data gathered from all the plots (partly presented in Gr. I and II) are compared, it appears that a tolerance for manganese may be due:

1. to a weak absorption of manganese, preventing any risk of injury through an excess. This could be noted in oats, mustard, mangold and perhaps white clover and vine.

2. To a strong tolerance within the plant, such as appears to be true for tobacco, flax, strawberry, potatoe and probably broad bean. No investigation has been made of the way in which this excess of manganese is distributed within the plant.

The lowest manganese content which appeared to induce visible injury and the highest content still allowing a healthy development of the various young crops are recorded in Table II and are indicated by horizontal lines in Gr. I and II. The actual threshold value will occur between these levels. Whilst for beans the upper level was as already shown, very constant in successive years (Gr. I), the levels for vetch and lucerne were much lower in 1948 than in 1949. For these crops no definite value could be decided upon. For the other crops data are available for one year only.

TABLE II

Lowest content of manganese in young affected plants and highest in young healthy plants

Crop plant	Content of manganese 1 : 1 000,000 dry weight in							
	1946		1947		1948		1949	
	aff.	healthy	aff.	healthy	aff.	healthy	aff.	healthy
Bean (Prinsesse boon)	1210	536	1211	642	1167	670	1104	904
Bean (Bruine boon)			1589	1142	1092	1036	922	855
Vetch					496	363	1177	975
Lucerne					477	452	1083	648
Red Clover							1300	910

The maxima reached in all the liming series by the various crop plants are recorded in Table III. When the content in the AS plots

exceeded this amount, this figure is added in parenthesis. Since in the later years all crops in these plots were somewhat depressed in growth, even when they showed no visible signs of injury, it is possible that other factors acting together with manganese excess may have affected the crops; hence these figures are less valid than the others.

TABLE III

Maximum content of manganese in young plants in the series of limed plots (AS plots in parenthesis)

Crop plant	Content of manganese 1 : 1 000.000 dry weight in				
	1944	1946	1947	1948	1949
Bean Prinsesse-boon		**3510**	**3665**	2702 (4216)	1709
Boon Bruine boon	**2052**	2290 (2981)	2670	**3047**	2236 (2535)
Vetch			2709	1552	**2678** (3276)
Lucerne				1047 (1152)	1225 (2429)
Red clover					1905
Tobacco			2936		
Potato Eigenheimer	896	1717 (2225)			
Flax					2137
Mangold					947
Mustard				598 (901)	
Strawberry		1641			
Oats	370				

When the data for the various crops are compared, it appears that the highest manganese content occurred in *Phaseolus* in four of the years. Presumably the great susceptibility of this species may be connected with the ease by which manganese is absorbed.

When the data furnished by the solution cultures are compared, it is seen that the tolerance for manganese of the various plants was generally less in von der Crone solution than in solution II. No definite conclusion can be drawn, however, since the experimental data are not sufficient in number and temperature conditions may have played a part because comparative experiments were not always run simultaneously.

When the contents of plants grown in von der Crone solution in the presence of a similar supply of $MnSO_4$ are compared the amounts may vary greatly (Table IV).

TABLE IV

Manganese content of foliage in solution cultures		
Mg Mn/l	5 mg	10 mg
Lucerne	2243 A	—
Vetch	1524 A	—
Mangold	1424	1650
Flax	1261	—
White clover	813	—
Broad beans	787, 641	1489, 1766
Pea	392	783, 837
French bean	—	2938 A
Lettuce	—	2036 SA

A = affected; SA = slightly affected.

These data corroborate the results of field experiments in as much as tolerant plants may be recognized as such by means of solution cultures. They do not allow a conclusion as to the actual cause of this tolerance in the field, when, as in the case of broad bean, both a low uptake in the presence of 5 mg and a fairly high one in the presence of 10 mg occurs.

4. THE ANTAGONISTICS OF MANGANESE

Constituents of the culture solution may influence the amount of manganese actually absorbed.

a. Literature.

Parberry[20]) noted an increased absorption of manganese, when magnesium was deficient. Hewitt[7]) has described reduced toxicity of manganese in the presence of calcium. Morris and Pierre[18]) failed to corroborate these results for *Lespedeza*. Millikan[16]) described an antagonistic action of molybdenum on manganese toxity in flax and (1948) in pea, cabbage and potato.

The antagonistic action of iron has, however, been studied more closely but with conflicting conclusions.

The first instance of injury caused by excess of magnanese in the soil was reported in Hawaï, where pineapples suffered injury on manganiferous soils.

Johnson[11a]) proved that excess of manganese made iron unavailable. He ascribed the effect to the oxidising action of MnO_2, the main form in which manganese occurs in Hawaian soil. The identity of the manganese induced injury with iron deficiency for pineapples has been well substantiated.

Somers and Shive[23]) in an extensive study of soy beans in streaming solution cultures were able to establish a distinct reciprocal antagonism between iron and manganese. They arrived at the conclusion that

it is the ratio of soluble Fe/Mn in the plant (optimum about 2) which determines the occurrence of the injury, not the absolute level of either element. In their opinion the symptoms caused by high soluble manganese and low iron are identical with those due to iron deficiency and conversely, low manganese will induce symptoms of iron excess. The manganese induced injury as described by them is, however, not identical with the injury Morris and Pierre [19]) induced in soy beans by excess of manganese. The latter scientists noted a clear difference between manganese-induced and iron-deficiency symptoms in soy beans.

Hopkins *et al* [10]). showed clearly by means of experiments with beans that iron could act as an antidote to manganese toxicity. Normal growth was observed in media containing high Fe/Mn ratios and no symptoms due to excess of iron could be found. Since these workers did not show whether iron deficiency could lead to symptoms similar to those produced in high manganese cultures their conclusion as to its induction in the latter cultures is not convincing.

Hewitt [7]) reported that manganese-induced injury in beans does not respond to a "painting" with ferric citrate. In [9]) he furnished arguments against the existence of a reciprocal action of iron and manganese.

Morris and Pierre [18]) working with *Lespedeza* established that the iron-manganese ratio in solutions was not a primary factor in the growth of the plant. Good growth was obtained over a wide range of iron-manganese ratios. The beneficial effect of iron in reducing manganese toxicity was due to a decrease in the manganese absorbed by the plant rather than to an increase in iron absorption. Iron deficiency symptoms were clearly different from those induced by manganese excess.

b. Experimental results.

Whilst the various investigators arrive at similar results in one respect, viz the antidotic effect of iron on the toxicity of manganese, in other respects their conclusions are conflicting. Neither the toxic affect of large doses of iron nor the identity of the manganese-induced injury with iron deficiency as claimed by Somers and Shive could be corroborated by other investigations.

When I started these investigations in 1943 I set up some observational solution cultures with beans, omitting iron. By this means a chlorosis all over the leaves was induced, which gradually turned a paperwhite, the veins only remaining green. This injury differed sharply from the injury induced by manganese excess.

Incidentally, however, I made observations which may throw some light on the existing controversy.

Beans grown in a greenhouse in summer (June 22th–July 21th 1949) in von der Crone solution provided with 7.5 and 10 mg Mn/l showed symptoms differing from those with which I was fami-

liar; the leaves were of a much more even white than usual. Areas of these leaves were painted with a $\frac{1}{4}\%$ $FeSO_4$ solution and after two days these areas had responded with a bright green colour. Apparently manganese excess had here induced an injury, differing from the usual one and identical with the iron deficiency injury noted by Somer and Shive.

On August 8th a fresh series of bean plants was set up in von der Crone solution with varying amounts of $MnSO_4$ in an unheated warehouse. When the first leaves developed a period of great heat set in and again in several vessels with 5–15 mg Mn/l the iron deficiency symptoms occurred (Fig. 24) which could be cured by means of $FeSO_4$ (Fig. 25). In some plants both types of injury could be noted.

These are merely preliminary observations and the actual conditions causing this phenomenon have not yet been worked out. Perhaps in former years it may have occurred sporadically in bean cultures during periods of great heat, but was then overlooked.

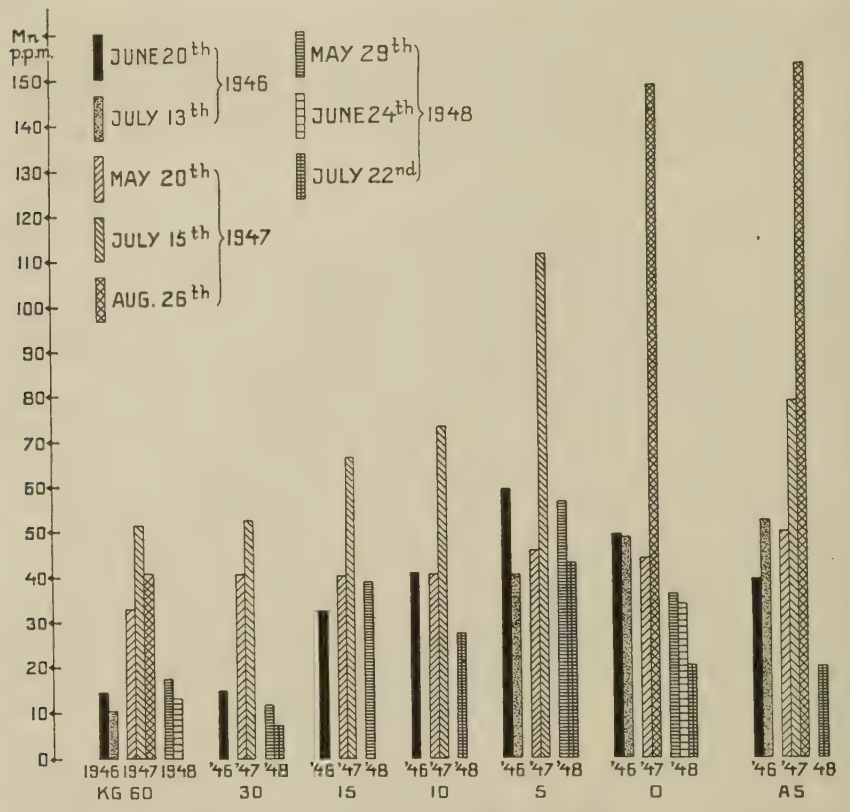
In the experimental field, however, where plants are mature before the hot weather begins, it has never been observed. It is possible that the fact that research on plant nutrition is usually carried out under greenhouse conditions may have caused the existing controversy.

As has already been mentioned symptoms of iron deficiency have been induced in mangold cultures by manganese excess. Since these plants were grown side by side with the beans in the experiment of August 8th-September 7th external conditions might have played their part here as well.

5. MANGANESE IN THE SOIL.

Another question which requires discussion is the effect of soil variation upon the availability of manganese. It is well known that the pH of the soil and its oxido-reduction status determine the form in which manganese will occur. Manganese may exist in varying degrees of oxidation; in neutral or alkaline soils the soluble bivalent manganous salts undergo rapid oxidation to form the insoluble MnO_2 and other higher oxides. Doubtless microorganisms also play a part in this proces.

The injury from which plants are found to suffer in acid soils may be due to various causes. In the Netherlands the main cause has



Gr. III. Exchangeable manganese estimated in soil from the plots in Series I and AS_1.

been recognized as magnesium deficiency (S m i t and M u l d e r 1942). Nevertheless, in some soils excess of manganese appears to cause damage to susceptible crops. The question is therefore posed as to, whether it might be possible to measure the manganese available to the plant directly in the soil. The very extensive literature on this subject, which concern chiefly the methods suitable for determining manganese deficiency in soil, will be passed over. A simple method has been applied by H e i n t z e [6]) in Rothamsted to the estimation of excess of manganese and by it I have estimated the exchangeable manganese in soil.

5 g soil taken up in 100 ml ½ molar $Ca(NO_3)_2$ solution is shaken for one hour and then filtered. In the filtrate manganese is estimated. The soil tested has never been stored for more than 3 days.

When the investigation of the soil was started in 1946 a correlation seemed to exist. When, however, soil samples were gathered under greatly varying weather conditions in later years, amounts estimated in a same plot appeared to vary widely. In Gr. III the amounts estimated in three successive summers in plots I_0, I_{60} and AS_1 show striking differences. For instance in 1947, in an exceedingly dry summer, the amounts were very high whilst in the very rainy summer of 1948 low amounts were recorded, because the manganese fraction estimated in the filtrate had apparently been washed out. In Table V the contents of exchangeable manganese in limed and

TABLE V

Amounts of exchangeable manganese in soil compared with the content of manganese in the bean plants in ppm.

Plot	I_0 kg				I_{60} kg				AS			
	Soil filtrate		Plant foliage		Soil filtrate		Plant foliage		Soil filtrate		Plant foliage	
Year	Mn	date	Mn	date	Mn	date	Mn	date	Mn	date	Mn	date
1946	49	20/6	3510	27/6	14.6	20/6	277	27/6	39	20/6	2840	27/6
1947	13	20/5	3081	6/6	33.3	20/5	518	6/6	49.6	29/5	3394	6/6
					51.4	15/7	84	20/7	78	15/6	2702	9/7
1948	36.3	29/5	—	—	17.7	29/5						
	34	24/6	2442	16/6	13.5	24/6	113	16/6	19.9	24/6	4216	16/6

unlimed plots are recorded along with the contents of the bean plants grown on them.

Whilst in 1947 in plot I_{60} the exchangeable manganese exceeds the quantity in I_0 1946, the plants in the former plot were quite healthy and even contained the lowest amount of manganese recorded.

For a reliable detection of an injurious amount of available manganese in the soil this method proves of small value.

A culture test in soil, such as has been described on page 200 with "Prinsesseboon" as an indicator plant, will furnish a more reliable answer: within a few weeks young plants cultivated in a soil sample will, whenever available manganese is present in excess, present the characteristic pattern of injury.

Testing acid soils by these means might prove of practical importance and might help to disentangle the intricate complex termed as "soil acidity".

I am indebted to Professor Dr. J. Smit for granting me the opportunity of carrying out this investigation in the Laboratorium

voor Microbiologie, to Dr. K. T. Wieringa for good advice and interest in the problem, to the Technical staff of the Laboratory for their helpfulness, to Professor E. C. Wassink for the opportunity of cultivating plants in the greenhouse of the Laboratorium voor Plantenphysiologisch Onderzoek and to the Organisation T.N.O. (Applied Scientific Research) for providing me with a warehouse.

SUMMARY

1. In unlimed or sparsely limed experimental plots *Phaseolus vulgaris* appeared seriously affected. Young leaves showed a characteristic pattern of injury and might completely fail. Less injured plants might recover. These symptoms could be reproduced in solution cultures supplied with excess of $MnSO_4$.

2. During several years the manganese content of beans growing in these plots has been estimated. In healthy young leaves the value ranged from 40–940 Mn ppm, in affected leaves from 1104–4261. The lowest level of injured leaves varied in 1946–1949 between 1211–1104 ppm.

3. In solution cultures (von der Crone) 5 mg Mn/l induced the symptoms. At high temperature bean plants appeared to tolerate a high content in the foliage without visible injury.

4. The composition of the culture solution acts greatly on the amount of manganese taken up by the plant. A difference in the proportion of the manganese supplied which remains in solution cannot explain this action.

5. Strict proof of the injury being due to manganese excess was furnished by the similarity of symptoms induced in beans, when cultured in a vessel with "sick" soil and in the same soil provided with $CaCO_3$ and $MnSO_4$ Acidity was here eliminated as causal agent.

6. In experimental plots *Vicia sativa* and *Medicago sativa* appeared very susceptible and showed characteristic signs of injury. *Trifolium pratense* showed slight symptoms and a final recovery. *Trifolium repens* merely lagged in initial development. Solution cultures corroborated the field tests.

7. Mangold was depressed in growth in more acid plots. Solution cultures did not corroborate the field tests.

8. Flax, potatoes, tobacco, mustard, oats and strawberry appeared tolerant in the field tests.

9. Peas, *Vicia faba* and vine appeared insensitive in solution cultures. Lettuce showed a slight injury in the presence of a large dose of $MnSO_4$.

10. When the contents of manganese in the foliage are compared, tolerance for manganese appears either to be due to a weak absorption or to a strong tolerance within the plant. The high sensitiveness of beans may be due to a very strong absorption.

11. Manganese induced symptoms of beans in the field are not identical with those induced by iron deficiency.

In solution cultures, however, at high temperature manganese excess was seen to induce iron deficiency.

12. The amount of exchangeable manganese in soil appeared to vary widely in plots investigated over a number of years. No correlation was found between these values and the content of manganese in the foliage of beans.

13. As a soil test for manganese excess *Phaseolus vulgaris* may prove of value as a test organism.

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pH TOLERANCE AND POLYPLOIDY IN ANGIOSPERMS

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It is well known that Angiosperm ecology, the grouping of species according to their habitat, has demonstrated the occurrence of ecological communities such as sand, salt-marsh, chalk and heath floras. Yet cyto-ecology, the relation of plant chromosomes and plant ecology, is virtually an unexplored field in studies on plant and soil inter-relationships.

This paper considers the concept of a relation between chromosomes and local plant distribution, as compared with the wider interpretation of chromosomes and plant geography already reviewed by Cain (1944). It reviews some aspects of diploidy and polyploidy of flowering plant species in relation to their soil acidity preferences and gives special consideration to calcicolous species.

REVIEW OF THE LITERATURE

Polyploids may differ in several ways from the diploids which produce them. Kostoff (1940) divided tetraploids which had arisen experimentally into three groups according to their direction of variability. Size of plastids seemed to be the only character not influenced by chromosome doubling. After comparing some of the physical attributes of autopolyploids and allopolyploids with diploids, Kostoff concluded that many of these examples indicate that polyploids which appear in nature can survive and, because they have the power to vary, can be acted upon by natural selection.

A study of the positive and negative aspects of the physiological and chemical changes accompanying polyploidy are important for a better understanding of the competition between diploid and polyploid species in nature. In these respects Noggle (1946) has studied the physiology of polyploidy to see how increase in chromosome number affects mineral composition, vitamin content and

other nutritive components of plants. Löve and Löve (1943) studied the geographical distribution of polyploids of Angiosperms and demonstrated the trend for increased frequency of polyploids towards the north and also in higher, *i.e.* alpine, regions. Their evidence supported the theory that polyploids have greater hardiness or better adaptibility in more severe climates than diploids. They found, too, that polyploidy is more frequent among monocotyledons than among dicotyledons.

Crane and Gairdner (1923), from studies with British species of *Cochlearia*, were early to realise that the ecological distribution of closely related species might be linked up with chromosomal differences. Recently the more local, *i.e.* ecological, aspects of polyploidy have been studied by Lovkvist (1947), who found that even in the same field plants of *Cardamine pratensis* were distributed according to their ploidy; diploids grew towards higher parts and polyploids congregated towards lower parts of the field. Lovkvist considered that this regional distribution indicated a relationship between chromosome number and water content of the soil. It might, however, have been due to differences in pH tolerances of the diploids and polyploids, although no estimate of pH in the various parts of the field were made. Similarly Hancock (1942) has found different ecological preferences in *Galium palustre*, diploids grew in damp places which dried out in summer, while large octoploids occurred in permanently damp or wet zones.

Rohweder (1937) found that one of his diploid *Dianthus* species did not grew too well on calcareous soil (although most *Dianthus* species favour alkaline soil) but the plants thrived better following doubling of their chromosomes. Many other tetraploid plants grew in the calcareous soils he investigated, perhaps indicating preference of polyploids for higher pH conditions. Noguti *et al.* (1940) had shown that tetraploids contain greater amounts of metallic elements and less of acid elements than diploids. Rohweder therefore thought it possible that chromosome doubling increases ability of plants to utilize certain soil constituents; this has considerable economic interest.

Polya (1948) examined thirty-four plants from Hungarian alkali soils and found twenty-four were diploids and eleven polyploids, these being all tetraploid except one species which was hexaploid. This makes interesting comparison with his finding in

fifteen salt-loving halophytes that only three were polyploids and of nineteen species facultatively halophil eight were polyploids. On these grounds Polya concluded that polyploidy and the halophytic character are not correlated.

In view of these observations and to test whether polyploids are more confined to chalky regions, an investigation was made to study the distribution of diploids and polyploids in relation to their pH requirements.

ATTRIBUTING pH VALUES TO CHROMOSOME NUMBERS

There are two ways of determining pH requirements of diploids and polyploids. One is to test the pH range of a number of diploid species, artificially double the chromosome number in each and then test the pH range of their autotetraploids. This is laborious and difficult. The other way is to take a sample of flowering plants whose pH ranges are known and then re-classify them according to ploidy. The plants can also be subdivided into monocotyledons and dicotyledons. This method permits investigation of more species but does not allow comparison between auto- and allo-polyploids.

Spurway (1941) listed the pH preferences of over seven hundred species of flowering plants and for each gave the optimum pH range most favourably for growth, provided other soil conditions also are favourable. In general pH of soils runs from 3.5 to 8.5 and for most cultivated soils is from 4.5 to 7.5. Spurway's list was used throughout as the source for pH tolerance. The ploidy was determined from chromosome numbers and basic x values given in *The Chromosome Atlas* (Darlington and Janaki, 1945) and *The Merton Catalogue* and *Supplement* (Maude, 1940; Rutland, 1941). Spurway had classified his list in alphabetical order of the popular North American names but comparisons were made only on latin names. In some instances, if the latin name given by Spurway was not identical with that given in *The Chromosome Atlas* the species was not included even though the same plant was probably being compared. Also where there was doubt as to whether a species could have been diploid or polyploid it was omitted from the tables. From such comparisons it was possible to construct a table containing the total number of plants falling into each of the various combinations at 0.5 intervals, the pH range being taken from 3.0 to 8.5.

THE DISTRIBUTION OF pH RANGES

Table I gives the number of dicot and monocot species classified for ploidy and pH range. All classes of polyploids have been grouped together. An analysis of variance was made on the data, Table II.

TABLE I

Angiosperms classified according to optimum pH range. (Rows with no species have been omitted)

Optimum pH range *)	Dicots				Monocots			
	Diploids		Polyploids		Diploids		Polyploids	
	No.	%	No.	%	No.	%	No.	%
3.0—5.0	1	0.24	0	0.00	0	0.00	0	0.00
3.5—5.0	1	0.24	0	0.00	0	0.00	0	0.00
4.0—5.0	4	0.96	2	1.09	4	5.80	3	4.84
4.0—5.5	0	0.00	4	2.17	0	0.00	0	0.00
4.0—6.0	3	0.72	0	0.00	0	0.00	0	0.00
4.0—6.5	0	0.00	1	0.54	0	0.00	0	0.00
4.0—7.0	2	0.48	0	0.00	1	1.45	0	0.00
4.0—7.5	0	0.00	0	0.00	1	1.45	0	0.00
4.0—8.0	0	0.00	1	0.54	0	0.00	1	1.61
4.5—5.0	5	1.20	2	1.09	0	0.00	0	0.00
4.5—5.5	5	1.20	2	1.09	1	1.45	0	0.00
4.5—6.0	17	4.08	4	2.17	4	5.80	3	4.84
4.5—6.5	2	0.48	0	0.00	1	1.45	0	0.00
4.5—7.0	0	0.00	2	1.09	1	1.45	0	0.00
4.5—8.0	0	0.00	1	0.54	0	0.00	0	0.00
5.0—6.0	49	11.75	35	19.02	13	18.84	9	14.52
5.0—6.5	28	6.71	7	3.80	5	7.24	4	6.45
5.0—7.0	4	0.96	1	0.54	1	1.45	0	0.00
5.0—7.5	17	4.08	6	3.26	1	1.45	3	4.84
5.0—8.0	4	0.96	1	0.54	0	0.00	0	0.00
5.5—6.0	1	0.24	0	0.00	0	0.00	0	0.00
5.5—6.5	20	4.80	5	2.72	2	2.90	3	4.84
5.5—7.0	27	6.47	10	5.43	3	4.35	1	1.61
5.5—7.5	12	2.88	7	3.80	0	0.00	2	3.23
5.5—8.0	0	0.00	0	0.00	2	2.90	3	4.84
6.0—6.5	3	0.72	1	0.54	0	0.00	0	0.00
6.0—7.0	41	9.83	18	9.78	11	15.94	9	14.52
6.0—7.5	88	21.10	38	20.65	2	2.90	7	11.29
6.0—8.0	65	15.59	27	14.67	12	17.39	7	11.29
6.5—7.0	1	0.24	0	0.00	0	0.00	0	0.00
6.5—7.5	13	3.11	9	4.89	3	4.35	1	1.61
6.5—8.0	4	0.96	0	0.00	0	0.00	1	1.61
7.0—7.5	0	0.00	0	0.00	1	1.45	1	1.61
7.0—8.0	0	0.00	0	0.00	0	0.00	3	4.84
7.0—8.5	0	0.00	0	0.00	0	0.00	1	1.61
Total	417	100	184	100	69	100	62	100

*) Ranges in which there were no plants are omitted from the Table but included in the analysis of variance.

As each figure in Table I represents only a small sample of the total flowering plants which could fall into that particular group, the analysis was made on data transformed according to the equation $X' = \sqrt{x + 0.5}$ (Snedecor, 1946) and included all combinations for pH ranging from 3.0 to 8.5.

The results of the analysis are given in Table II. All main items

TABLE II

Analysis of variance of transformed data in Table I

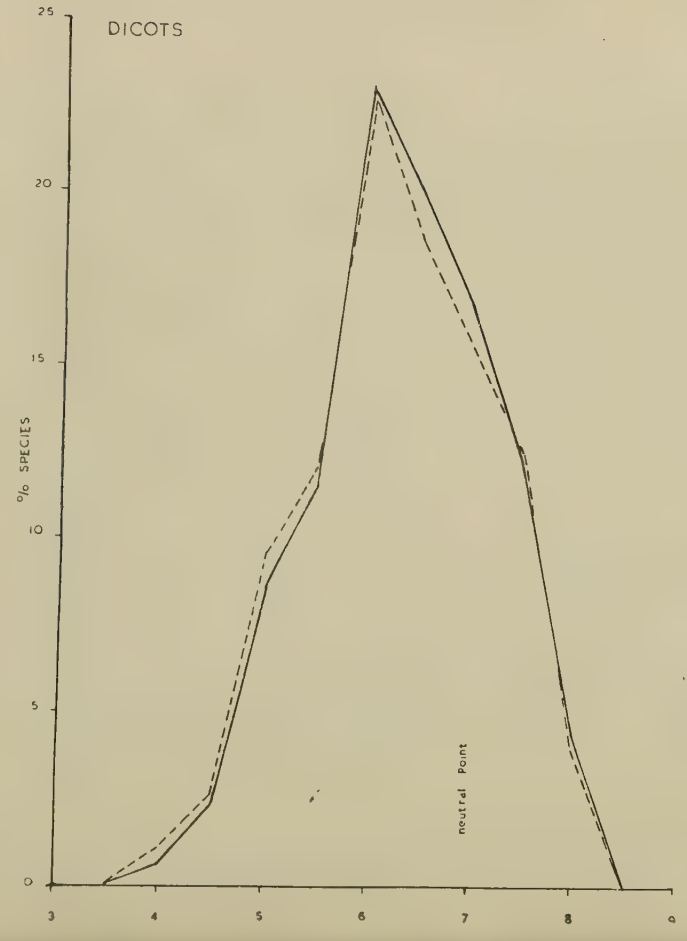
Item	*S.S*	*N*	*m.s.*	*t*	*V.R.*	*P*
pH range	291.27	65	4.48		15.32	Highly sig.
Group	38.73	1	38.73	11.50		,, ,,
Ploidy	7.28	1	7.28	4.98		,, ,,
Range-Group	69.57	65	1.07		3.66	,, ,,
Range-Ploidy	20.35	65	0.31		1.07	Not sig.
Group-Ploidy	5.94	1	5.94	4.50		Highly sig.
Remainder	19.01	65	0.29			
Total	452.15	263				

are significant. Interest, however, lies in the first-order interactions. Here the item 'range-group' is highly significant indicating that on the whole, monocots and dicots have different pH preferences. The item 'range-ploidy' is, on the other hand, not significant; thus statistically it is not demonstrated with the material used that ploidy in general modifies significantly the pH preferences of species. The item 'group-ploidy' indicates that monocots and dicots have different frequencies of polyploid species and this is already known.

Frequency of optimum pH

As the data of Table I give distribution for range only and do not take into consideration the numbers involved at any particular pH value, they were therefore re-arranged to show how many species of each of the four types have their optimum pH passing through any one pH value. Adjusted data are given in Table III and illustrated in Figure 1.

In both dicots and monocots most species have their optimum pH at 6.0, and in dicots there are no differences in pH references of species of either ploidy. On the other hand, as Figure 1*b* shows, there is a clear tendency for polyploid monocots to shift in the direction of a higher optimum pH value *i.e.* a trend towards a more alkaline environment.



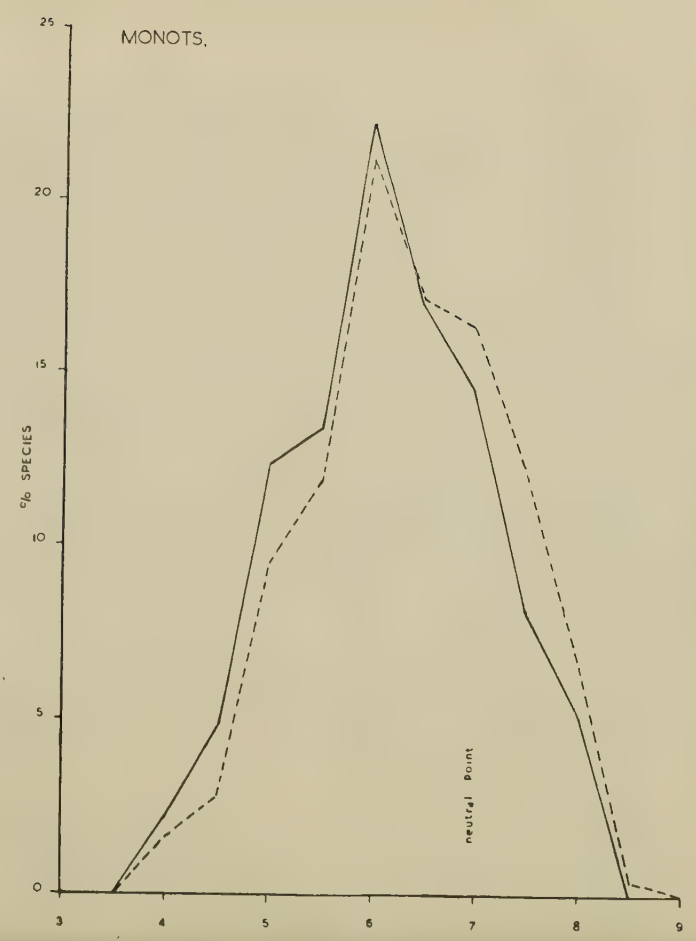


Fig. 1. Frequency of species of flowering plants plotted against values of pH. Left: Dicots; Right: Monocots.

TABLE III

Angiosperms re-classified according to number whose optimum range pass through a particular pH value

No. with optimum range passing through pH	Dicot				Monocot			
	Diploid		Polyploid		Diploid		Polyploid	
	actual	%	actual	%	actual	%	actual	%
3.0	1	0.06	0	0.00	0	0.00	0	0.00
3.5	2	0.12	0	0.00	0	0.00	0	0.00
4.0	11	0.66	8	1.11	6	2.23	4	1.64
4.5	40	2.42	19	2.62	13	4.83	7	2.87
5.0	142	8.58	69	9.53	33	12.27	23	9.43
5.5	191	11.54	87	12.02	36	13.38	29	11.89
6.0	383	23.14	165	22.79	60	22.30	52	21.31
6.5	331	20.00	135	18.65	46	17.10	42	17.21
7.0	278	16.80	121	16.71	39	14.50	40	16.39
7.5	203	12.27	90	12.43	22	8.18	30	12.30
8.0	73	4.41	30	4.14	14	5.20	16	6.56
8.5	0	0.00	0	0.00	0	0.00	1	0.41
Total	1655	100	724	100	269	100	244	100

Optimum pH ranges

As all species have a *pH range*, however limited, this is perhaps a better method of indicating pH tolerances.

Hence if the data are re-grouped into the number of species that have their *optimum ranges* falling into units of 1 pH difference, as in Table IV, several differences within and between groups emerge. As Figure 2 indicates, optimum range of the greatest number of species lies between 6.0 and 6.9 of both groups, yet although there

TABLE IV

Angiosperms re-classified according to their optimum pH tange

No. of species in optimum pH range	Dicot				Monocot			
	Diploid		Polyploid		Diploid		Polyploid	
	actual	%	actual	%	actual	%	actual	%
3.0—3.9	3	0.18	0	0.00	0	0.00	0	0.00
4.0—4.9	51	3.08	27	3.73	19	7.06	11	4.51
5.0—5.9	333	20.12	156	21.55	69	25.65	52	21.31
6.0—6.9	714	43.14	300	41.44	106	39.41	94	38.52
7.0—7.9	481	29.06	211	29.14	61	22.68	70	28.69
8.0—8.9	73	4.41	30	4.14	14	5.20	17	6.97

are no monocots with the optimum range group 3.0—3.9, like there is a small group of dicots, the monocots have a higher proportion of species, on the whole, in the optimum range group 4.0—4.9 and

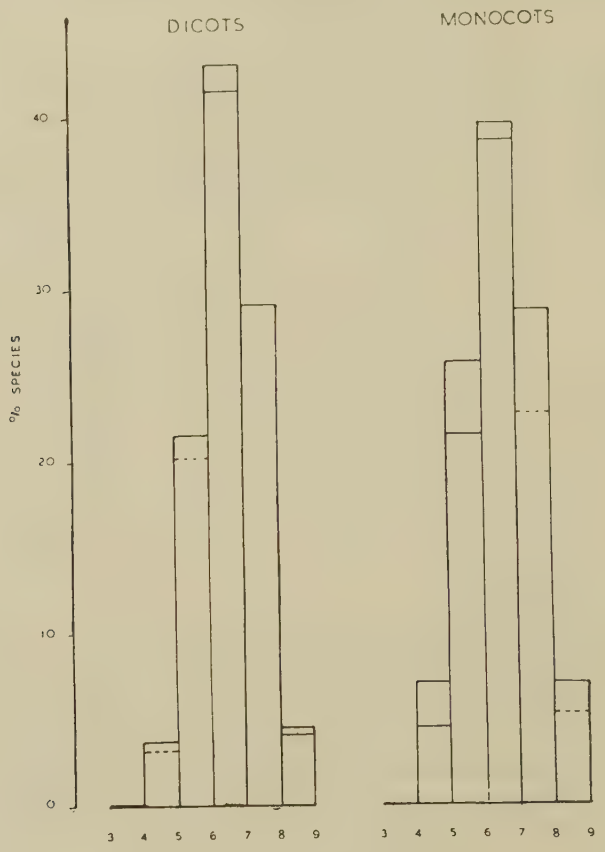


Fig. 2. Frequency of flowering plant species plotted against optimum range in pH units. Broken lines indicate that there are less diploids than polyloids.

8.0—8.9 than do the dicots. In the dicots, ploidy is of small significance, but in the monocots it is of importance as again illustrated by a somewhat higher proportion or polyploids to diploids whose optimum ranges lie between 7.0 to 8.9.

Mean pH distribution

Similar evidence for this is shown from the data in Table V. Here the data have been calculated on the basis of *mean pH values* for each range.

In dicots ploidy plays no significant role (Fig. 3). In monocots,

Fig. 3. Frequency of flowering plant species plotted against mean pH. Top: Dicots; Bottom: Monocots. Diploids: ——— Polyploids: – – – –.

where the number of species on which the calculations have been made are considerably less, the graphs are somewhat irregular. It is clear however that in dicots there are at least two peaks at mean pH 5.5 and 6.75, *i.e.* there is a distinctly acid group and a group just on the acid side of neutrality. The acid group could be accounted for by inclusion in S p u r w a y's list of such well-known horticultural groups like *Ericaceae*.

TABLE V

Angiosperms re-classified according to mean pH tolerances

No. of species with mean pH	Dicot				Monocot			
	Diploid		Polyploid		Diploid		Polyploid	
	actual	%	actual	%	actual	%	actual	%
4.00	1	0.24	0	0.00	0	0.00	0	0.00
4.25	1	0.24	0	0.00	0	0.00	0	0.00
4.50	4	0.96	2	1.09	4	5.80	3	4.84
4.75	5	1.20	6	3.26	0	0.00	0	0.00
5.00	8	1.92	2	1.09	1	1.45	0	0.00
5.25	17	4.08	5	2.72	4	5.80	3	4.84
5.50	53	12.71	35	19.02	15	21.74	9	14.52
5.75	29	6.95	9	4.89	7	10.14	4	6.45
6.00	24	5.76	7	3.80	3	4.35	4	6.45
6.25	47	11.27	18	9.78	4	5.80	4	6.45
6.50	57	13.67	26	14.13	11	15.94	11	17.42
6.75	89	21.34	38	20.65	4	5.80	10	16.13
7.00	78	18.71	36	19.57	15	21.74	8	12.90
7.25	4	0.96	0	0.00	1	1.45	2	3.23
7.50	0	0.00	0	0.00	0	0.00	3	4.84
7.75	0	0.00	0	0.00	0	0.00	1	1.61
Total	417	100	184	100	69	100	62	100

In the monocot diploids the situation is somewhat similar with peaks at 5.5 and 7.0 and additional smaller peaks at 4.5 and 6.5. In monocot polyploids the major peaks are at 4.5, 5.5 and 6.5, but no peak at 7.0. Yet there is another small peak at 7.5, well outside the range of all other groups. This again indicates that some monocot polyploids may be found tolerating highly alkaline ecological situations where dicots and diploid monocots have not yet established themselves.

pH range differences

Further information which can be extracted from the data is that concerning the *amount of difference* in pH range, expressed in 0.5 of a pH unit. This will indicate whether polyploidy and group influence

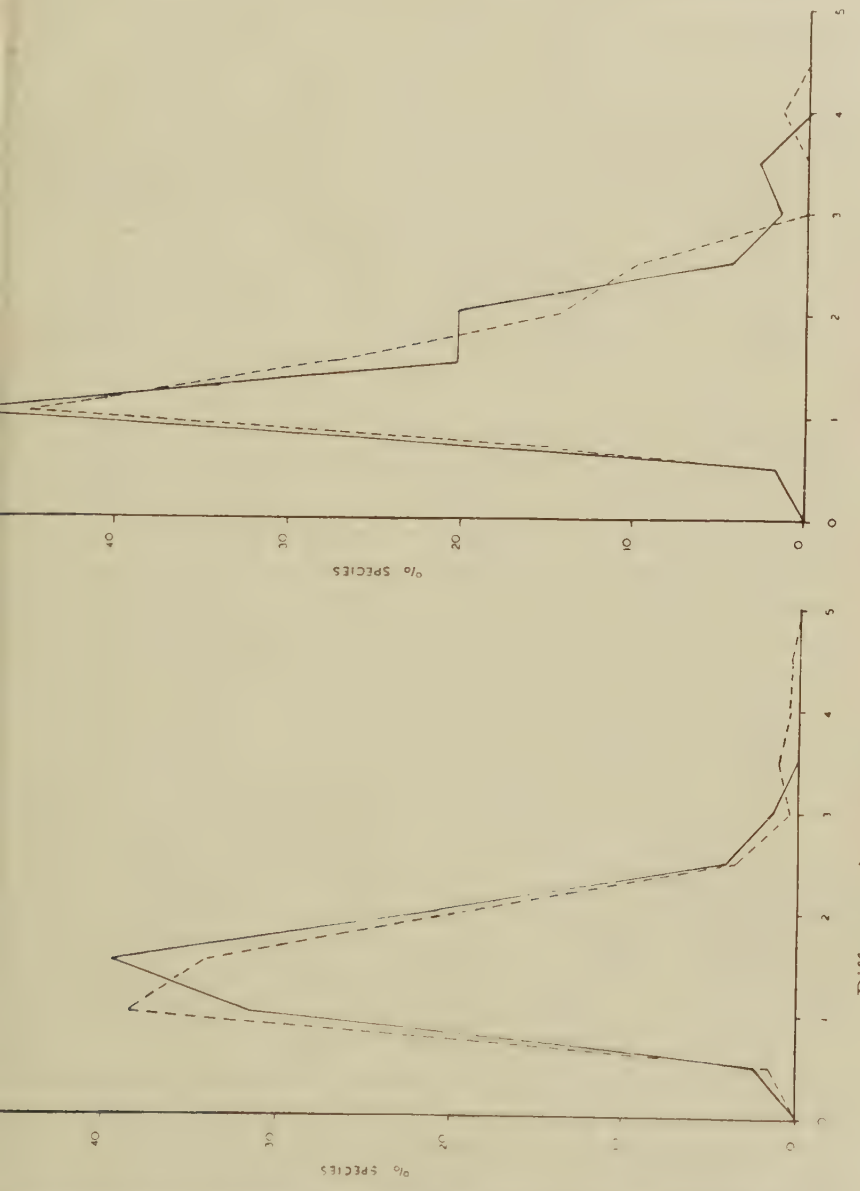


Fig. 4. Frequency of flowering plant species plotted against differences in pH range. Left: Dicots; Right: Monocots. Diploids: ————. Polyploids: – – – – –.

the extent of the range. Such data are provided in Table VI. From Figure 4 it is also seen that among the dicots the majority of polyploids have a pH range difference of 1 unit while the peak for the diploids is 1.5. Also there are a few polyploids with a higher range

than the diploids, *i.e.* some polyploids can tolerate wider extremes than diploids.

TABLE VI

Angiosperms re-classified according to their pH range differences								
No. of species with pH range difference	Dicot				Monocot			
	Diploid		Polyploid		Diploid		Polyploid	
	actual	%	actual	%	actual	%	actual	%
0.5	10	2.40	3	1.63	1	1.45	1	1.61
1.0	132	31.65	71	38.59	34	49.28	28	45.16
1.5	165	39.57	63	34.24	14	20.29	17	27.42
2.0	87	20.86	35	19.02	14	20.29	9	14.52
2.5	17	4.08	7	3.80	3	4.35	6	9.68
3.0	6	1.44	1	0.54	1	1.45	0	0.00
3.5	0	0.00	2	1.08	2	2.90	0	0.00
4.0	0	0.00	1	0.54	0	0.00	1	1.61
4.5	0	0.00	1	0.54	0	0.00	0	0.00

In monocots the diploid and polyploid species both have maximum number of species with a range of 1.0 pH difference but it is not so obvious that polyploids can have a wider range than the diploids. Comparisons of the two figures do not indicate that there are especial differences between monocots and dicots with regard to the relative frequency of species and their differences in pH range.

Removing Papilionaceae from the list

Some Angiosperm families are restricted ecologically through their possessing bacterial root nodules. As distribution of these nodules might in itself be influenced by soil acidity, the pH tolerances of the diploid dicot family *Papilionaceae* were therefore considered separately. The pH tolerance ranges of thirty-three species are given in Table VII. It is seen that most species fall into the 6.0–7.5 range and extend over 4.5 to 7.5 which is also that for the majority of dicot diploids. In general it appears that families themselves ecologically restricted by their root nodules are not likely, on the average, to have unusual pH tolerances compared with families lacking them.

TABLE VII

pH tolerance range of diploid species in the dicot family *Papilionaceae* possessing root nodules	
pH tolerance *) range	No. of species
4.5—6.0	1
4.5—6.5	2
5.0—6.0	3
5.0—6.5	3
5.0—7.5	1
5.5—6.5	3
5.5—7.5	1
6.0—7.0	5
6.0—7.5	10
6.5—7.5	2

*) Those with no species areomitted.

DISCUSSION

The problem of the inter-relations of soil pH and plants was considered at some length by Small (1945). In determining pH optimum range much depends on other influences such as base content, climatic humidity, aeration; soils cannot always be treated with the simplicity of laboratory cultures. Salisbury (1920) also has emphasized that artificial laboratory experiments to determine what chemical and physical conditions species will tolerate are no criterion of the success of a species in competition with others under the same conditions or under natural conditions.

Small (1946) placed acidiphilous plants in the pH range 3.5–4.8. He also provided lists of acidiphilous, acid tolerant, amphi-tolerant mesophilous (which grow under a wide range of conditions), alka-tolerant and alkaliphilous plants which occur in the British flora. In the alkaliphilous group the only monocot is *Poa compressa* which is a high polyploid. All of the five alka-tolerant and alkaliphilous species of pasture grasses listed by Small are polyploids, the only acid tolerant species for comparison being the diploid *Cynosurus cristatus*. But judgment on this very limited number of species must be reserved as *Agrostis tenuis* and *A. canana* are acid-tolerant yet polyploid.

Webb and Hart (1945) have suggested that the calcicole habit must be largely determined by differential survival in an environment that is universally unfavourable but that the balance between environment and competitive factors must be exceedingly delicate in determining whether the habit be calcicole (chalk-loving), calcifuge or indifferent. They also discuss the significance of the often extreme dryness of most calcareous soils. It is possible that some calcicolous species *e.g. Carlina vulgaris* are really species preferring dry soils. As Salisbury (1920) pointed out, the soil condition for a plant in the wild is to enable it to withstand pressure of competition, even slight edaphic changes or distinctions may profoundly modify the character of vegetation. Plants on calcareous soils may be there without real preference for such a soil, being there in absence of competition. For example, *Achillea atrata* and *A. moschata* thrive on siliceous or calcareous soils but when competing together *A. atrata* is calcicole and *A. moschata* is silicole. Unfortunately their chromosome numbers are undetermined so comparison of

the cyto-ecological relations is not possible. Similar conditions are known for *Pinus*. Such problems must be assessed when comparing the ecological habitats of species and before relating them to chromosome number.

Now the natural flora of the calcareous soil in Britain has a high proportion of legumes, due to conditions being favourable for nitrogen fixing organisms *e.g. Rhizobium radicicola*. Calcicolous flora exhibit xerophytic characters, and there all gradations in tolerance, restriction depending upon rigour of competition or other factors. It is at the climatic limits of a species range, whether geographical or altitudinal, that soil preferences become more clearly defined and it is here that cyto-ecological investigations should be concentrated.

Small (1946) found that *Rhizobium radicicola*, which is symbiotic, varied with the host plant in form and behaviour, and the above findings show that this is also true for the pH preferences of the species affected, as deletion of the *Papilionaceae*, a group with bacterial root nodules, does not affect the general trend of Table I.

Stebbins (1940), discussing polyploid complexes, found that most diploid species have relatively restricted distribution, polyploids being the most widespread, *e.g.* in *Crepis*. Stebbins (1938) also found that polyploidy was most frequent in herbaceous perennials and relatively infrequent in woody plants and in annuals, so this must be considered in cyto-ecological studies between species. In some monocot families, *e.g. Cyperaceae*, *Juncaceae* and *Iridaceae*, the presence of polyploidy is obscured by the large number of aneuploids or non-multiple series of chromosomes found in them. Some allopolyploids, such as *Spartina Townsendii* in Britain, have different geographical ranges from those of their diploid parents suggesting they are subjected to a different selective activity of the environment. Stebbins was also able to show that polyploids are dominant in regions which have only recently opened to occupation by plants, or which have been subjected to great climatic or other environmental changes, while the diploids tend to occupy the older, more stable habitats. This agrees with the view that newly colonised alkaline areas might be expected to possess polyploid monocots as invaders.

According to Sax (1936) tetraploids may have a more northern range than the diploids, or a more alpine distribution and a greater

distribution along the sea coast. He considered that the polyploids originated before the extension of the species range and because they are hardier extend into more extreme environments. A similar situation apparently holds true for monocot polyploids with regard to their soil pH tolerance but does not apply to the dicots. T i s c h l e r (1946) also concluded that polyploids have a higher selection and a higher colonizing capacity than diploids. He also found that in Europe the percentage of polyploids increases from south to north with the percentage of monocot polyploids substantially higher than that of polyploid dicots in the equivalent regions. Such conclusions, then, on plant geography are in agreement with our theoretical deductions on the ecology of angiosperm groups and their ploidy in relation to their pH tolerances. The stage is now reached where the principles of plant geography in relation to polyploidy can now, with profit, be applied to local ecological floras.

Acknowledgements. The writer wishes to thank Mr. M. B. C r a n e F.R.S. for his interest in, and support of, this study.

Summary

1. A Table of Angiosperms was constructed with each species classified according to cotyledon group, ploidy (based on *The Chromosome Atlas*) and pH tolerance (based on S p u r w a y's List).

2. On the whole, monocots and dicots have different pH tolerance preferences but there was no statistically significant difference for the interaction of ploidy with pH tolerances.

3. In dicots there are no differences in pH preferences of either ploidy, while in monocots polyploids shift in the direction of a higher optimum pH value.

4. Most dicots and monocots have an optimum pH range between 6.0 to 6.9. There is no difference for ploidy in dicots but in monocots there is a somewhat higher proportion of polyploids to diploids whose optimum pH ranges lie between 7.0 to 8.9.

5. Ploidy in dicots plays no significant role in distribution according to mean pH values but there is indication that monocot polyploids may be found tolerating highly alkaline situations not yet colonized by dicots or diploid monocots.

6. The most frequent amount of difference in pH range in diploid dicots is 1.5 units and 1.0 for polyploid dicots and both types of monocots.

7. Distribution in respect to pH of the soil of families with bacterial root nodules is not affected by the presence of nodules.

8. The literature on the relation of polyploidy to plant nutrition, soil

requirements and plant distribution is reviewed. The study of chromosome number of plant species in relation to their ecological habitat needs further investigation.

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METABOLISM OF PLANTS AFFECTED WITH LIME-INDUCED CHLOROSIS (CALCIOSE)

I. NITROGEN METABOLISM

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Yellowing of the foliage of plants growing on soils rich in lime is generally explained as due to iron deficiency, the iron being unavailable because of soil alkalinity. Moreover the iron present in such chlorotic leaves is considered to be, in large part, in an inactive form. This explanation is not supported, however, by the results of numerous tissue analyses made by the writer, and by the findings of several other investigators.

Attention may be directed, first of all, to the fact that the normal flora on limestone soils, termed calciphilic species by some investigators, is not chlorotic and the iron assimilation of such species may be normal, whereas calciphobic species, including certain cultivated species may not find such soils favorable for their normal growth. In previous reports (7, 8, 9, 10, 11, 12) attention was directed to the fact that abnormal metabolism and abnormal biochemical content of tissues accompanies chlorosis of some species of plants on limed soils. Inhibition or delay in production of chlorophyll may not in itself be regarded as the cause of this disease, for it may be induced by such different factors as unfavorable temperature, excess or deficiency of water, excess or deficiency of mineral salts, poisoning by certain substances, etc. Moreover the yellowing from lime-induced chlorosis may differ from that produced in consequence of a deficiency of iron. By means of biochemical analyses, it has been found possible to determine the character of the disease and to trace its progressive development. The writer has, therefore, made systematic analyses of the leaves of normal and chlorotic plants, giving consideration to their nitrogen metabolism, to car-

bohydrate and organic acid metabolism, and to ash content. The present report is concerned only with the nitrogen metabolism of healthy and diseased plants. The results of analyses for other constituents will appear in subsequent reports.

MATERIALS AND METHODS

The present investigations were made using plants growing near Baden and Humpldskirchen, suburbs of Vienna, Austria. They involved 17 species of plants grown in fields and gardens during the years 1941 to 1944. In some cases, series of samples were collected during the entire period; and, in others, they embraced a shorter period, samples being taken for analysis at some chosen stage of development of chlorosis.

A large number of leaves was collected for each sample. The sample was divided immediately into two parts. The larger part was subjected to a temperature of 90°C. This portion was used in determining total nitrogen, by Kjeldahl's method, minerals, salts, starch, and certain other constituents. The smaller part was placed in a wide-mouthed jar, and the leaves were quickly killed by exposure to chloroform vapor. The sap was then promptly expressed and subjected to chemical analyses. Preliminary tests had established that no appreciable enzymatic changes occurred in such samples during the period from collection to fixation.

Other methods of fixation were discarded in favor of the use of chloroform. Previous work (7) had shown that high temperature causes the precipitation of calcium salts. A y e r s [1]) confirmed this finding, noting that calcium and sulphur are precipitated when extracted sap is heated in an autoclave.

Fixation by freezing might have been employed, but S c h l e n k e r [19]) has recently shown that only $^1/_8$ of the asparagine remains after freezing.

If plant tissues are extracted in water, a larger amount of substances may appear than was actually present in the sap. It was found, for example, that 8 to 10 times more calcium may be extracted from grape leaves than occurs in the sap, and similarly that soluble nitrogen may occur in larger amount.

The various nitrogen fractions in the expressed sap were determined as follows: Total soluble nitrogen was determined by the

method of Kjeldahl; protein nitrogen, after precipitation with trichloracetic acid, was also determined by Kjeldahl's method; ammonium was measured, at 46°C, after alkalinization with sodium carbonate and sodium chloride; amides, after boiling for 2 hours with H_2SO_4 and then aerating; residual nitrogenous materials, by precipitation with phosphotungstic acid followed by digestion by the Kjeldahl method; proteins were calculated by difference between total and soluble nitrogen, using as a basis the ratio of one ml of sap and one g of dry weight.

Results of analyses are arbitrarily expressed on the basis of dry weight of leaf tissue. As will be shown, healthy and chlorotic leaves differ in proportional water content and in content of essentially all other constituents. The inherent difficulties, when making comparisons by use of dry weight or of green weight, seem, therefore, to be unavoidable.

TOTAL NITROGEN

In apples

Three different varieties of apples were employed in analyses made in 1943. Many of the trees in the garden from which the samples were taken were suffering to a greater or less extent from chlorosis, and some of them were so severely affected that they perished later. The samples were taken from selected trees, an apparently healthy tree and an affected one of each kind. The affected Yellow Noble apple had yellow-green foliage, and it bore a few small fruits; the normal Yellow Bellflower fruited well but a few of its leaves were spotted with yellowish green, whereas the affected tree bore only a few small fruits and its foliage was markedly yellow. Only the veins were green on the affected Canada Reinette tree and it bore no fruit.

Total nitrogen determinations were made from samples collected May 11, June 1, June 22, July 29, Aug. 21, Sept. 14 and Oct. 5 with the results as presented graphically in Fig. 1. It is obvious from Fig. 1 that, in spring, the total nitrogen in chlorotic foliage of apple exceeded that in normal leaves but it decreased continuously throughout the season, so that, by autumn, chlorotic foliage contained a lesser amount of nitrogen than normal foliage. Ultimately, the leaves possessed approximately half or a lesser amount of nitrogen than was present in the spring.

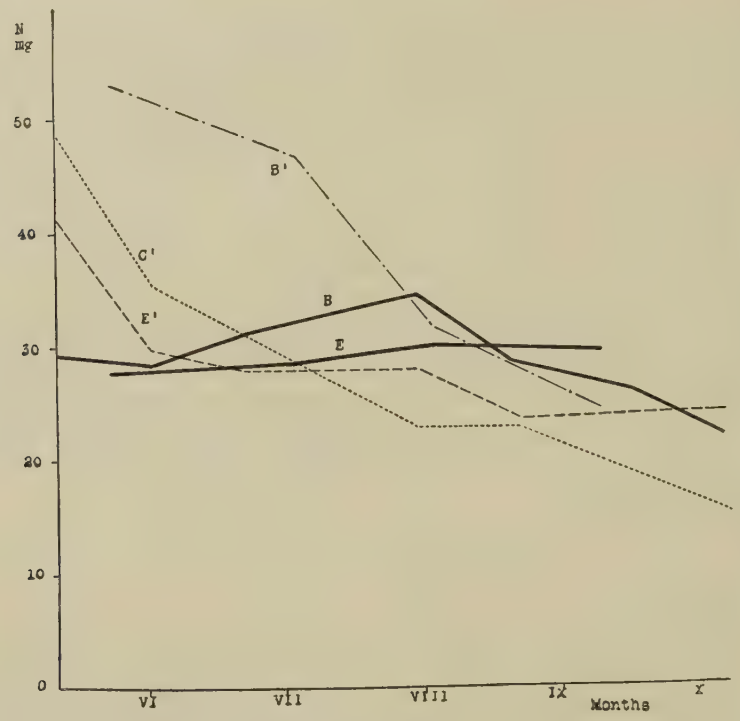

Fig. 1. *Pyrus malus*. Total nitrogen in leaves. Yellow Bellflower: B — green, B′ — chlorotic plants. Yellow Noble apple: E — green, E′ — chlorotic plants. Canada Reinette: C′ — chlorotic plants.

In cherries

Similar analyses were made of two varieties of *Prunus avium*, namely „Gelbe Knorpel" and „Johannes". The latter kind was the more seriously affected, since some of the trees died and others became defoliated by midsummer. The normal, healthy tree from which leaf samples were taken was a thrifty, well-developed tree with large leaves, only a few of which had light green spots. Analyses of leaves from this healthy tree, made May 21, 1943 and May 25, 1944, showed a total nitrogen content of 28.8 and 29.2 mg of nitrogen respectively, whereas the corresponding values at these dates for Gelbe Knorpel were 36.7 and 39.4 mg, respectively, and for Johannes 41.2 and 60.2 mg respectively. Considerable differences in total nitrogen in normal and chlorotic cherries and in normal and chlorotic apples therefore exist in the early growth in spring.

In grapes

During three seasons, observations and analyses were made of chlorotic and normal grapes grown in a school garden in Humpldskirchen. The varieties included Rotgipfler, Rheinreisling, Spätrot, Neubirger, and Blaufrankish. All are not equally subject to lime-induced chlorosis, the first being especially sensitive.

As anticipated, in healthy grapes the nitrogen compounds undergo from year to year regular alterations during the season. There are maxima and minima which delimit the variations induced by external factors. If the variations exceed these limits, it indicates that the plants are abnormal. The results of these analyses of grape foliage are shown in Fig. 2. The heavy line, A, portrays the average

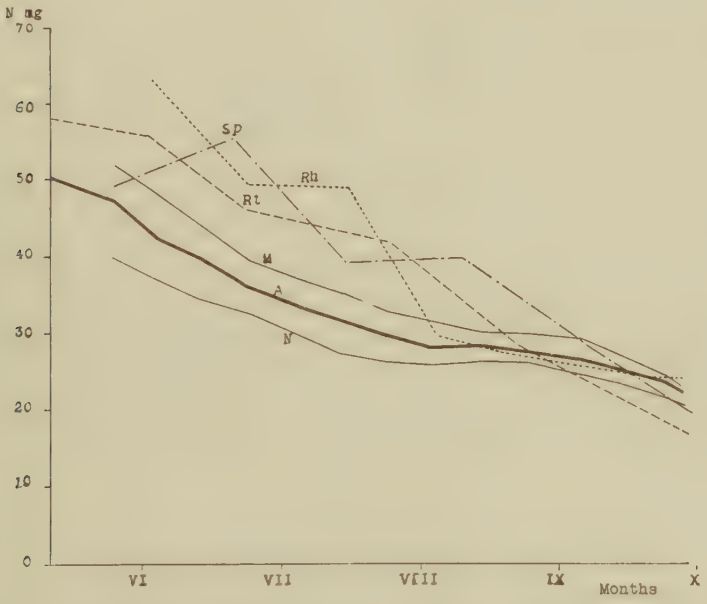

Fig. 2. *Vitis vinifera*. Total nitrogen in leaves. Healthy plants: A — the average content, M — the maximum, N — the minimum of nitrogen. Diseased leaves: Rt — Rotgipfler 1943, Rh — Rheinriesling 1942, Sp — Spätrot 1944.

total nitrogen content; and the light lines, M and N, represent maxima and minima, respectively. It may be noted that all chlorotic grapes had a nitrogen content in excess of that of normal plants and

that there was a gradual decline from spring to autumn, there being about half as much in autumn as in spring. The greatest reduction occurred while the leaves were young and new tissues were being formed.

If the chlorotic plants tend to recover toward the end of summer, then the nitrogen curves tend to approach those of healthy plants, as seen in Fig. 2.

In lupine

On July 19, lupine seeds were sown on two sites, one richer in lime and more alkaline than the other. On August 11, when the plants had developed to the extent of having 6—7 leaves, samples were taken for the first analysis. A second analysis was made September 2. The total nitrogen per 1 g of dry weight from the field having plants with green leaves, only slightly chlorotic, on these two dates was 51.5 mg and 40.0 mg respectively; while the corresponding samples from the other field having yellow-green foliage was 58.0 mg and 27.1 mg respectively. The decline in total nitrogen was, therefore, approximately 22% and 54% in the same period of time.

Johannes cherry, which is very susceptible to chlorosis, may not be equally affected throughout the entire tree, and by midsummer the leaves on part of the branches may have become twisted, dry, and may have been shed. Analysis of the leaves of such a tree, made in 1943, showed the following differences in milligrams of total nitrogen:

TABLE I

Total nitrogen of normal and chlorotic cherry foliage in mg/g dry wt. 1943

Dates of analysis	May 21	June 12	July 19	Aug. 6	Aug. 30
Leaves green	28.8	29.3	26.7	22.1	21.0
Leaves yellow-green . .	39.0	33.0	31.2	29.6	26.4
Leaves yellow-white . .	41.2	44.0	36.6	—	—

A somewhat comparable tree, that perished in late August, 1944, was found to have the following amounts of total nitrogen when its leaves were subjected to analysis in comparison with those of a normal tree:

TABLE II

Total nitrogen of normal and chlorotic cherry trees in mg/g dry wt 1944				
Dates of analysis	May 25	July 10	Aug. 10	Sept. 8
Normal tree	29.2	26.3	24.4	17.1
Chlorotic tree				
Leaves yellow-green	39.4	29.4	30.5	28.4
Leaves yellow-white	60.2	38.7	44.2	—

In other species

In 1942, samples were taken of two groups of bushes of *Sambucus nigra*. Those of one group were normally green and of the other were visibly chlorotic. The results of analyses revealed the following amounts of total nitrogen:

TABLE III

Total nitrogen of the foliage of *Sambucus nigra* in mg/g dry wt					
Dates of analysis	June 26	July 29	Aug. 27	Sept. 14	Oct. 1
Normal leaves	34.2	30.3	27.2	21.2	17.5
Chlorotic leaves	31.8	43.3	40.6	34.8	33.3

Again young leaves in spring contained the largest amount of nitrogen, and there was a gradual decrease as the season progressed. Also the chlorotic leaves contained the larger amounts.

Other analyses involved the foliage of *Syringa vulgaris*, grown on limestone soil. One selected group of plants exhibited no evidence of chlorosis, whereas the symptoms were clearly expressed in the other two groups, one more pronounced than the other. On analysis, the following results were obtained:

TABLE IV

Total nitrogen of *Syringa vulgaris* foliage in mg/g dry wt				
Dates of analysis	June 26	July 29	Aug. 27	Oct. 2
Green leaves	26.0	25.4	21.8	17.2
Slightly chlorotic leaves	28.6	23.9	17.4	—
Markedly chlorotic leaves	34.3	27.5	27.4	27.1

The decrease in nitrogen in slightly affected plants most nearly resembles that in normal plants, but marked decreases do not occur in severely chlorotic plants.

Other species analyzed include *Ailanthus glandulosa*, *Robinia*

pseudoacacia, Fraxinus excelsior, Cydonia oblonga and *Lupinus lutea.* In each of these species, the total nitrogen was greater in chlorotic leaves than in leaves of the same age from normal plants. Moreover, the decrease in total nitrogen in chlorotic plants tended to be greater in the early part of the season, but continued to decline, extending into autumn. Also the changes in normal plants throughout the growing season tended to be relatively small, i.e., the total nitrogen tended toward uniformity and stability.

As a result of the foregoing analyses and observations, the question arises as to the source of the large proportion of nitrogen in chlorotic leaves, whether from the soil, whether transferred from other organs, as from stored reserves, or whether it is synthesized *de novo.* It was observed that all apples, cherries, and grapes growing near Baden and Humpldskirchen in rainy, humid springs were chlorotic, so that it was quite impossible to find a single normally green plant. On the other hand, in other seasons, it was difficult to find a typically chlorotic tree or vine, or there were, at most, only a few that appeared abnormal. Consideration was, therefore, given to the mobility of reserve nitrogen. This was done by using yellow lupine, as shown in the following tabulation of analytic results:

TABLE V

Total nitrogen in yellow lupine foliage in mg/g dry wt				
	Elapsed period from date of planting-days			
Tissues analyzed	20	27	30	44
Cotyledons	39.2	46.1	36.6	21.0
First pair of leaves	47.6	49.1	32.5	28.5
Upper leaves	44.0	61.5	57.2	35.0

It is apparent from the data in Table V that some of the nitrogen present in the cotyledons may have moved into the first pair of true leaves and thence it moved upward into the leaves formed later.

SOLUBLE NITROGEN

Preliminary analyses on the ratio of soluble nitrogen to total nitrogen in chlorotic plants revealed that soluble nitrogen increases greatly in diseased plants. To determine more exactly these relationships, series of analyses were made throughout the growing season and involved several species of plants, principally apples, cherries, and grapes.

In apples

As previously indicated, three species of apples were used, Yellow Noble, Bellflower, and Reinette. Not only were comparisons made of chlorotic and normal foliage on trees grown near each other, but, in addition, analyses were made of healthy foliage taken from apple trees grown near Franzinbad, in northern Austria, where the soils were acid and lime was lacking. The soluble nitrogen in these healthy trees from Franzinbad was quite identical in amount with that in the selected normal trees. The data on soluble nitrogen in apple foliage are shown graphically in Figure 3. It is apparent that

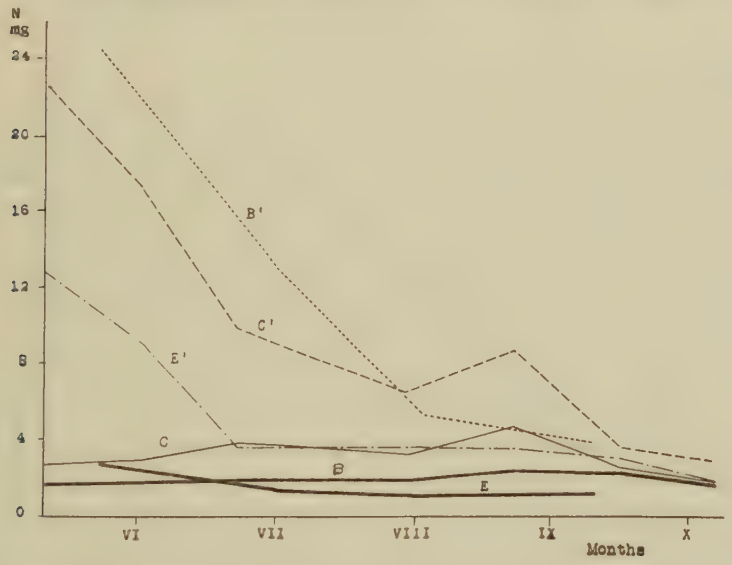

Fig. 3. *Pyrus malus*. Soluble nitrogen. Noble apple: E — green, E′ — yellow. Bellflower: B — green, B′ — yellow. Canada Reinette: C — green, C′ — yellow plants.

the amount of soluble nitrogen remains essentially constant throughout the season in healthy plants. On the other hand, there may be approximately 7 to 15 times as much in chlorotic foliage in spring, but by the end of summer the amount had declined to the extent that it approached that of healthy foliage.

In cherries

In 1943 and 1944, similar determinations were made of soluble nitrogen in two varieties of cherry, Johannes, a variety very sus-

ceptible to chlorosis, and Knorpel, one that possesses considerable resistance. The samples included green, apparently healthy leaves, yellow-green leaves, and yellow-white leaves. Some of the trees were so severely diseased as to bear yellow-white foliage, and they shed their leaves and perished by mid-summer. Again, as with apples, the amount of soluble nitrogen in chlorotic foliage of cherry is greater in spring than at the end of summer. The magnitude of these differences is of the order of 5 to 15 times, depending upon susceptibility and the severity of the disease. Also the soluble nitrogen of the less seriously affected foliage tends to approach that of normal foliage by the end of the growing season.

In grapes

Determinations of soluble nitrogen in grape foliage were made over a period of three seasons. From these analyses, graphs were constructed to show the maximum amounts, the minimum amounts, and the average amounts of soluble nitrogen in healthy plants at different times throughout the growing period. These data are portrayed in Fig. 4. In comparison are shown separately the results of analyses of chlorotic foliage of three varieties. The outstanding feature from these analyses is the considerably greater amount of soluble nitrogen in chlorotic foliage at the beginning of the growing season and the sharp decline throughout summer and fall, when the content of chlorotic and normal leaves tends to be approximately the same. When the leaves were young, approximately 10% of the total nitrogen in normal foliage was soluble; whereas, in chlorotic leaves, approximately 30% of the total nitrogen was soluble.

In other species

Similar comparative studies, involving the changes in content of soluble nitrogen in healthy and chlorotic leaves, were made, using *Sambucus nigra, Fraxinus excelsior, Ailanthus glandulosa, Cydonia oblonga, Rosa canina, Cornus mas, C. sanguinea* and *Lupinus lutea* and *L. alba*. The results, with each species showed the same large differences in soluble nitrogen between diseased and healthy foliage early in the season and the same type of decline, as the season advanced, as was found with apples, cherries, and grapes. They showed also that, on a given date, the upper leaves contained more soluble nitrogen than did middle leaves, and the middle leaves more

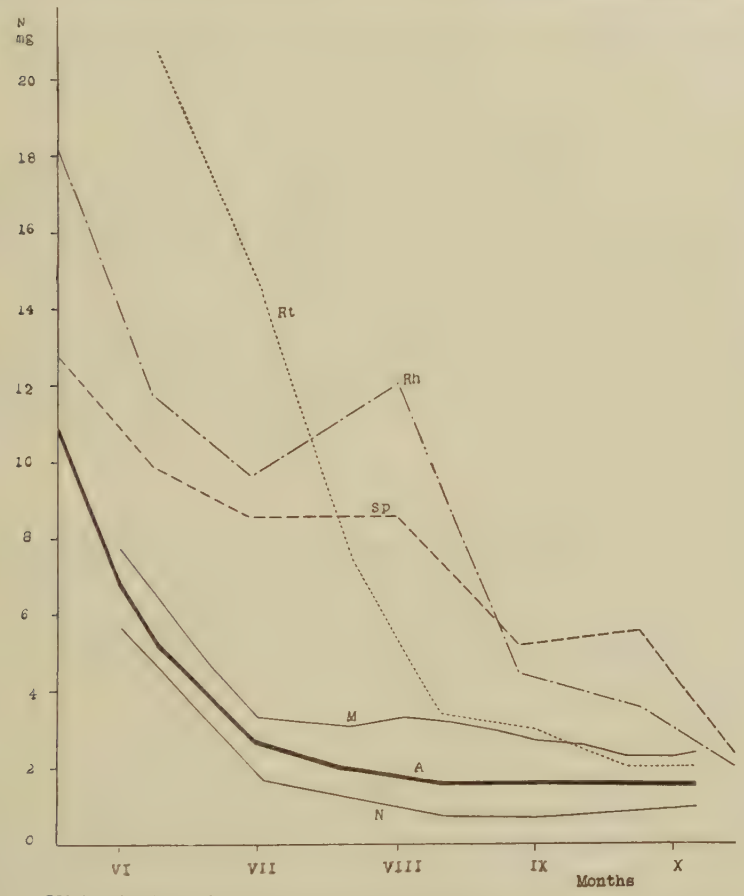

Fig. 4. *Vitis vinifera.* Soluble nitrogen. Healthy plants: A — average content, M — maximum, N — minimum. Diseased leaves: Sp — Spätrot 1943, Rh — Rheinriesling 1943, Rt — Rotgipfler 1942.

than the lower leaves, whether the plants were healthy or chlorotic. Regardless of the species of plant, moreover, the more severe the chlorosis, the greater the concentration of soluble nitrogen in the leaf tissues.

Chlorosis is known to be first manifest by a yellowing of the leaf tips and leaf margins. As the disease becomes more severe, the yellowing spreads to involve the tissues most distant from the principal veins and midribs and, at the same time, progresses also toward the leaf bases. Thus the tissues adjacent to the vascular system retain their green color longest. This pattern of disease de-

velopment may be interpreted to indicate that the gradual accumulation of soluble products also proceeds in the same fashion.

SOLUBLE NITROGENOUS SUBSTANCES

As regards soluble nitrogenous substances in cell sap, the determinations made included amino nitrogen, ammonium nitrogen, amid nitrogen, nitrogen bases, and protein nitrogen. It was found that ammonium nitrogen was equally present in small amount in healthy and diseased plants. In some instances, the other nitrogenous products were determined separately and, in others, collectively. As anticipated, amino acids were found to accumulate in actively growing tissues and may presumably be transformed ultimately into proteins.

Amino acids in grape

The characteristic changes in amino acid content of grape foliage, both of normal and chlorotic plants, were followed throughout a period of 3 years. Pertinent data from the analyses are summarized in Fig. 5, to show the ranges of maxima and minima for healthy grape foliage and the trends of changes in chlorotic plants of each of three varieties.

It may be observed that the amino acid content of chlorotic foliage is greater than that in healthy leaves, the differences being within the range of magnitude of approximately 3 to 7 times as much. One of the factors accounting for differences is severity of the disease, the larger amounts being in the most severely affected plants. In healthy plants, the amino acid content, which is greatest in young leaves, tends to become and to remain quite uniform throughout the entire growing season once equilibrium has been established.

Measurements of amino acid content of apple foliage were also made; and they showed that, at the beginning of the season, the differences in content between healthy and diseased leaves were greatest and gradually became less toward autumn.

Similar analyses of the changes in amino acid content of cherry leaves were made. Again, as with apples and grapes, the changes throughout the season in healthy trees were smaller than those in diseased trees. Amino acids were found in greatest amount in most severely chlorotic leaves, especially in Johannes cherry, which is

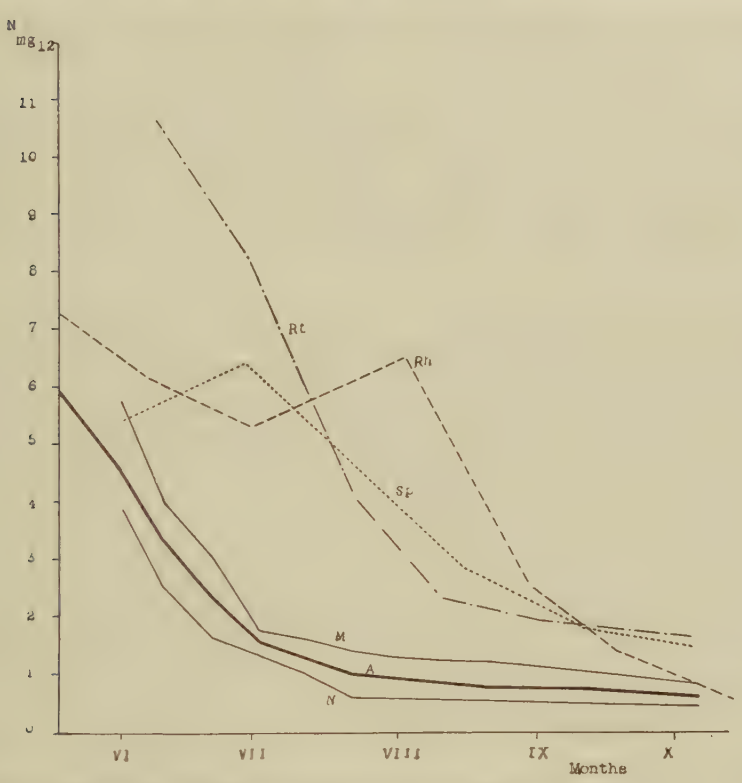

Fig. 5. *Vitis vinifera*. Nitrogen of amino acids. Normal plants: A — average content, M — the maximum, N — the minimum. Chlorotic plants: Sp — Spätrot 1944, Rt — Rotgipfler 1942, Rh — Rheinriesling, 1943.

highly susceptible to chlorosis. Amino acid determinations involving other species included *Lupinus luteus*, *L. albus*, *Syringa vulgaris*, *Fraxinus excelsior*, and *Ailanthus glandulosa*. In each of these species, chlorotic leaves had a larger content of amino acids than did normal leaves. For example, in *Syringa vulgaris*, there was about 3 times as much, in *Fraxinus excelsior* about 9 times as much, and in *Sambucus nigra* about 8 times as much. In general, therefore, chlorotic tissues are characteristically high in amino acids.

Nitrogenous bases and protein nitrogen

This fraction, in certain species, as *Lupinus luteus*, constituted a considerable portion of the soluble nitrogen, but in general it is less

than half that of the amino acid content. The results of analyses of the foliage of apple, cherry, lilac, and ash show similar relationships.

It was noted that chlorotic foliage consistently contained more nitrogenous bases and protein nitrogen than did normal foliage. There did not appear to be any regularity in trend, however, in this fraction throughout the season.

Residual nitrogen

There is included in this fraction that nitrogenous material represented by the difference between soluble nitrogen and the sum of all the other determined fractions. In some instances, there was no residual nitrogen, all of it being accounted for in the separate fractional analyses. In general, healthy plants contained little or none of this fraction; whereas there was always a considerable amount in chlorotic foliage. In some species, 40 to 50% or more of the soluble nitrogen may be included in this fraction. Data bearing on these relationships, as found by analyses of apple leaves, are assembled in Fig. 6. The importance of the differences in residual

Fig. 6. *Pyrus malus*. Remaining nitrogen. Green leaves: E — Noble apple, Chlorotic leaves: E′ — Noble apple, B′ — Bellflower, C′ — Canada Reinete.

nitrogen should be stressed, because it is believed that there may be in this fraction toxic nitrogenous compounds. Such nitrogen-containing compounds as normally appear during the synthesis of proteins or during their dissimilation should not be toxic to the plant. The accumulation in tissues of products included in the portion called residual nitrogen may conceivably disturb the physiological balance; and, hence, this fraction may be a contributory cause of chlorosis. At least, this hypothesis should be investigated further.

Determinations of residual nitrogen were also made, using cherry, lilac, Ailanthus, and lupines; and, in each species, the qualitative results approximated those with apple foliage. In lilac, the amount of this fraction exceeded that of the amino acids. In lupines the residual nitrogen appeared in greater amount in the upper leaves, coincidental with decrease in amount in cotyledons and in lower leaves.

INSOLUBLE NITROGEN

The largest part of this fraction is protein, and it includes the portion represented by the difference between total nitrogen and soluble nitrogen. Young leaves contain largest amounts of insoluble nitrogen. This insoluble nitrogen is utilized as the tissues mature, so that there is at first a sharp decline, after which the decrease proceeds gradually.

The insoluble nitrogen relationships in normal and chlorotic leaves of grape and apple are shown in Fig. 7, A and B.

It may be noted that the insoluble nitrogen increased during the first part of the season in Noble apple. A similar course of change was exhibited by cherry and lilac; whereas, in ash and in *Ailanthus*, the level of insoluble nitrogen remained uniform throughout the season. In general, there appears to exist a balance between synthesis of proteins and their assimilation. Such is not the case, however, in chlorotic plants.

Either through decline in rate of conversion of amino acids into proteins or through increase in rate of assimilation of proteins, there is produced an unbalance in the processes of synthesis and analysis. For example, in healthy apple leaves, approximately 90% of the total nitrogen exists at the end of the season in the form of proteins; whereas, in chlorotic leaves, approximately only 50% exists as proteins. Similarly, in normal cherries, 93% is protein, in yellow-green leaves, 68%, and in yellow-white leaves, 29%.

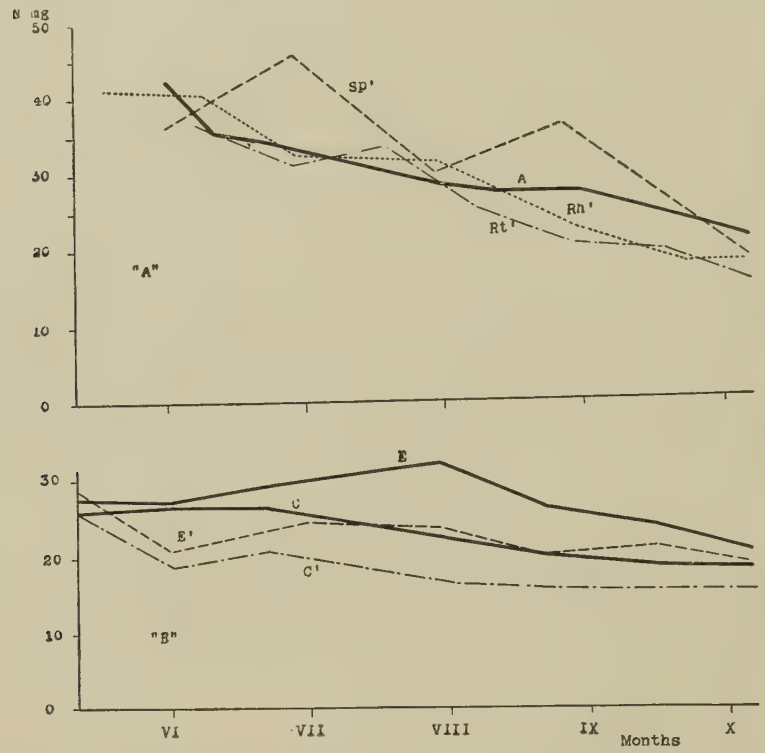

Fig. 7. Insoluble nitrogen. „A" *Vitis vinifera*: A — green leaves, Sp′, Rh′, Rt′, Spätrot, Rheinriesling, Rotgipfler — chlorotic leaves. „B" *Pyrus malus:* Noble apple: E — green, E′ — chlorotic leaves. Canada Reinette: C — green, C′ — chlorotic leaves.

Similarly, chlorotic lupines exhibited failure of establishment of balance between synthesis and assimilation of proteins. With them, as with the other plant species under observation, deviations in tendency toward adjustment of balance occurred throughout the season; and these deviations, dependent upon their direction, reflected either recovery or else a more serious stage of the disease. In chlorotic Rotgipfler grape, for example, three successive analyses showed a protein content of 69, 80 and 71% respectively, and those of Spätrot grape, 79, 83, and 76% respectively.

SUMMARY

The studies involved analyses of 17 species of plants and extended over a period of 4 years. From the massive volume of data that was aggregated in tables and graphs, only typical results have been selected for presentation.

As expected, regular alterations were found to occur during the course of the season in nitrogen metabolism of healthy plants. The same fluctuations occur at the same periods during different years. Curves may be drawn to represent maximal and minimal changes; and, when these limits are exceeded, the plants are abnormal.

In spring, the total nitrogen content of young leaves of healthy plants is highest, and it gradually decreases after the leaves are fully formed. The leaves of chlorotic plants in spring, however, have a considerably greater total nitrogen content, the more severe the chlorosis, the greater the content.

At the beginning of the season, there may be five to fifteen times as much soluble nitrogen in chlorotic leaves as in healthy ones. Since soluble nitrogen is rapidly assimilated normally, its content in healthy tissues remains at a low level.

By early summer there may be 0.5 to 1 mg of amino nitrogen in healthy tissues; whereas, in a corresponding weight of chlorotic tissues, there may be 3 to 7 mg.

Ammonium nitrogen in spring in normal and chlorotic tissues is similar and small in quantity.

Healthy leaves contain 0.2 to 0.3 mg per g of dry weight of amid nitrogen, whereas chlorotic leaves may contain 10 or more times as much.

Chlorotic leaves contain more soluble proteins and bases than do normal ones.

Residual nitrogenous compounds are absent in healthy foliage or are present in small amounts only, but may account for 40 to 65 per cent of the soluble nitrogen in chlorotic plants.

Approximately 94 to 96% of the total nitrogen in fully developed foliage is insoluble. When the leaves are young, 80 per cent may be insoluble; but, as growth proceeds, proteins are synthesized; and the norm is quickly attained. In chlorotic foliage, only 30 to 40 per cent of the soluble fraction may be synthesized into insoluble form.

The presence of abnormal amounts of nitrogenous substances, both soluble and insoluble, in the cell sap can be expected to interfere with the normal physiologic activity of such cells. Such disturbance of function is visibly expressed by interference with the formation of chlorophyll and other pigments, as characterizes chlorosis.

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THE EFFECTS OF DEFICIENCIES OF SEVERAL TRACE ELEMENTS ON TIMOTHY (*PHLEUM PRATENSE L.*) GROWN IN SOLUTION CULTURE

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The essentiality of various trace elements for growth of higher plants is now widely recognised. A great deal of work has been and is being done, using a wide range of materials and of methods. However, the individual need of any particular species of plant for each element has yet to be demonstrated, though the amount of evidence accumulated suggests more and more strongly that manganese, copper and zinc are almost certain to prove essential for all higher plants (Stiles [10]), Brenchley [4])). The essentiality of molybdenum remains more doubtful; a considerable number of accounts of trials with this element has been published, particularly in this country and Australia (Stephens and Oertel [11]), Anderson [1]), Anderson and Oertel [2]), Warington [15]), Fricke [5]), Wilson [16]), Hewitt and Jones [7]), Waring [14])).

Fricke describes the development and cure of molybdenum deficiency in oats. Stephens and Oertel report improved growth of ryegrass (*Lolium perenne L.*) in pots of soil, after addition of molybdenum. So far as the author is aware, however, this element has not yet been proved essential for any grass species.

On the whole, grasses have been rather neglected in the field of trace element investigations, though there is no reason to suppose that they differ qualitatively from other higher plants in their mineral requirements. The present investigation is concerned with timothy (*Phleum pratense L.*). This species was selected because it had previously been found to be more susceptible to deficiencies of major elements than other species used, while being an important one agriculturally. Beeson *et al* [3]) found it to be a low absorbing species for phosphorus and copper, though they place it in their "medium" group for cobalt and manganese. In the light of their

figures, the species might not be expected to be very sensitive to deficiencies; the different behaviour of their plants and ours was no doubt due to the different strains used.

MATERIALS AND METHODS

The variety of timothy used was Aberystwyth S.48. Plants were grown from seed; about 200 hulled seeds were selected and surface sterilized by washing for five minutes in 0.1% mercuric chloride plus a commercial spreader. They were then washed free of mercuric chloride, soaked overnight in distilled water and sown on purified talc moistened with dilute culture solution. When large enough to handle conveniently (i.e. with 2–3 leaves) seedlings were transferred to 100 ml conical flasks containing half strength culture solution, and when they began to tiller to their final culture vessels.

Wide necked conical flasks of one litre capacity were used as culture vessels. They were of pyrex glass, as was all the glassware used throughout the experiment: light was excluded from them by covers made of "Sisalkraft".

The plants were supported in the flasks by waxed corks until they were large enough to rest on the necks of the flasks without such support.

The chemicals used for supply of major nutrient elements were "Analar" salts and were repurified by the method of Steinberg as described by Stout and Arnon [12]). Trace element salts were not repurified, as the amounts used were very small. Water was redistilled in pyrex stills.

Culture solution was made up from molar stock solutions of calcium nitrate, magnesium sulphate and potassium phosphate (dibasic). Ten ml of each was used per litre of culture solution; the pH was adjusted to 6.5 by addition of glass -distilled hydrochloric acid.

The composition of this solution was thus:

Element	Amount in parts per million (p.p.m.)
N	280
P	310
K	780
Ca	400
Mg	240
S	320
pH 6.5.	

Trace element salts were kept as stock solutions at such concentrations that 1 ml per plant gave the required concentration. Amounts supplied (as parts per million of the final culture solution) were:

Element	Form	Amount
B	H_3BO_3	0.3 approx. (1 m.p.p. of H_3BO_3
Cu	$CuSO_4$	0.2
Mn	$MnSO_4$	0.3
Mo	Na_2MoO_4	0.01
Zn	$ZnSO_4$	0.1
Fe	$FeSO_4$ + tartaric acid	4.0 *)

*) As described by Stout and Arnon, 1 ml of iron solution (giving 1 p.p.m. to the solution) was added to the solution in each flask twice a week. Since solutions were changed once a fortnight, the total amount of iron added in that time amounted to 4 p.p.m., though the concentration was probably never much more than 1 p.p.m. at any time.

Solutions were changed once a fortnight. The flasks were topped up daily with glass-distilled water, after addition of iron when necessary.

The experiment was carried out in a greenhouse the glass of which was shaded with "Summer Cloud" throughout most of the time. The maximum air temperature reached was 100°F on August 13th and 14th, the minimum 40°F on June 16th.

Seeds were sown on May 5th, plants transferred to 100 ml flasks on June 22nd to their final culture vessels on July 17th. The long initial period in talc was due to the death of seedlings originally sown for the experiment on May 20th and transferred to flasks on June 1st; those which were finally used were unused plants left over from another experiment. For this reason there were no extra plants to be used as replacements.

Treatments used were absence of each of the five trace elements (boron, copper, manganese, molybdenum and zinc) singly, compared with controls which were given all trace elements. Five plants were used in each treatment, though final results were obtained from five plants only in the 'no copper,' 'no manganese' and 'no zinc' groups. One of the control plants was unhealthy from the start and never made normal growth, two of the 'no molybdenum' plants died as seedlings and one of the 'no boron' plants was damaged through accident and had to be discarded. No replacements were available, which made statistical treatment of the results more difficult.

RESULTS AND DISCUSSION

Characteristic symptoms developed of deficiencies of copper, zinc and manganese. Iron deficiency also occurred, presumably because the iron supply was inadequate. No symptoms of either boron or molybdenum deficiency appeared. Results are given and discussed below under the heading of each element in turn.

Copper

Symptoms of copper deficiency first appeared just over three weeks after the seedlings were transferred to culture solution. The leaves then turned very dark green and lost a little of their turgor, though not enough to be described as wilting. The colour, while darker than that of healthy leaves, was not the same as the dark bluish green characteristic of phosphorus deficiency. Neither growth of individual leaves nor tillering appeared to be affected at this stage.

Three weeks after this first appearance of colour symptoms, the youngest fully expanded leaves became necrotic at the tips in a characteristic manner. A distal region, including from a third to an eighth of the leaf's length rolled up from the edges inwards, then dried out and withered, and finally lost its colour and became whitish. Affected leaves thus appeared to be quite healthy towards the base but to end suddenly in a whitish thread. Younger leaves were then affected, their tips withering and dying before expansion and even before emergence from the subtending leaf, so that while the base of the leaf continued to grow, the tip remained enclosed in the next oldest leaf, and thus became looped. Sometimes as many as three successive leaves behaved in this way, giving the plants an untidy, tangled appearance (Fig. 1).

Growth did not appear to be affected until after one or more leaves on most tillers had reached the necrotic stage. After this very little growth occurred and the final weights of tops were significantly less than those of controls (Table I). Growth of roots was apparently unaffected, although the older roots of all plants died at the tips at the time leaf necrosis first appeared. Final root weights were not significantly different from those of control plants.

One plant produced flowers, but only one very small inflorescence.

These symptoms agree closely with those described by P i p e r [9])

1. Copper deficient plant, showing necrosis of leaf tips.

2. Control (L.) and zinc deficient (R) plants, showing stunted growth and necrosis of leaves caused by zinc deficiency.

3. Zinc deficient plant at a later stage than that in Fig. 2, showing necrotic patches on leaves.

4. L. to R. Control, manganese, copper and zinc deficient plants.

TABLE I

Mean dry weights of tops and roots of plants from different treatments

Treatment	Tops	Roots
Control	11.35 } ± 1.47	2.16 } ± 0.25
— Boron	11.10 }	2.47 }
— Copper	5.73 }	1.64 }
— Manganese	5.12 } ± 1.31	1.17 } ± 0.22
— Zinc	3.66 }	1.28 }
— Molybdenum	16.40 ± 1.69	3.10 ± 0.29
General Mean	8.14	1.85

Comparison of treatment means: — the difference in dry weight between two treatment means is judged to be significant if it is greater than twice the standard error of their difference.

e.g. comparing — B and — Mn

Mean difference = 11.10 — 5.12 = 5.98

2 × S.E. of mean difference $= 2\sqrt{1.47^2 + 1.31^2}$

$= 3.94$

the difference is therefore significant.

in *Lolium subulatum*. The only substantial difference was that he found flowering to be completely inhibited.

Zinc

The first appearance of zinc deficiency was not recorded until over a week later than that of copper, when the plants had been in culture solution for five weeks. Plants were then of a pale, greyish colour which gave them a dusty appearance. Although not in fact smaller than the control plants, they appeared somewhat stunted because their leaves were shorter relative to their width than those of normal plants. The leaves also grew stiffly upwards and did not bend over in the middle as readily as normal ones do (Fig. 2). Simultaneously with the appearance of these symptoms, some of the older leaves became chlorotic or necrotic. Chlorosis consisted of complete loss of colour, the affected patches turning white, but not yellow, then becoming necrotic. Other patches became necrotic without first becoming chlorotic. Younger leaves developed greyish-brown necrotic patches, usually near the middle. These were irregular in shape and in some cases spread across the whole width of the leaf, which then collapsed and the distal portion died. In other cases leaves died back from the tip (Fig. 3).

Three weeks after the first appearance of deficiency the plants seemed very much stunted, apparently not having grown at all

in the meantime. Dead parts remained a greyish brown colour, and no flower heads appeared on any of the plants.

Root growth was less than that of control plants, but the roots appeared quite normal and healthy. Dry weights of both tops and roots were significantly less than those of control plants.

Wood and Sibly [17]) working with oats, reported very similar symptoms, which they described as first appearing on the third or fourth leaf behind the growing point. They also observed purple colouration, which they consider to suggest a connection with deranged phosphorus metabolism. In the present investigation, purple colouration was observed on only one leaf of one plant, but in previous experiments with timothy purple colouration has not developed under conditions of phosphorus deficiency, so this difference may not be significant.

Manganese

Manganese deficient plants were late in showing clearly defined symptoms, though the plants grew less than controls and after six weeks of growth appeared weak; they also remained rather pale. Reduction in growth affected tillering, so that the plants appeared smaller than controls; it did not, however, affect leaf length as in zinc deficiency, though the leaves did appear rather narrower than those of control plants. They were also rather limp, as opposed to the stiff leaves of zinc deficient plants. The general effect was to give the plants a spindly appearance, as compared with zinc deficient plants, which appeared stunted.

Definite symptoms of manganese deficiency appeared only after thirteen weeks growth. The youngest leaves began to develop intervenal necroses in their middle regions, which spread both longitudinally and laterally, occasionally cutting right across the leaf, which then collapsed in the middle, leaving the tip green, as has been observed in oats.

Root growth appeared less than in controls; roots looked healthy though rather thin. Dry weights of both tops and roots were significantly less than those of controls.

Symptoms, while following the same general pattern as in oats, did not give the same appearance to the plants, since necrotic areas remained pale yellow-brown or white rather than grey, so that the "grey speck" condition typical in oats did not appear. The symptoms

bore some resemblance to those of zinc deficiency, but apart from the difference in growth the colour was different. In manganese deficient plants the leaves were pale yellowish green before necrosis appeared, whereas in zinc deficient ones they were pale greyish green. Also, necrotic areas in manganese deficient leaves were more strictly intervenal, spreading lengthways more readily than sideways, whereas in zinc deficient ones they were irregularly shaped and appeared to spread as readily sideways as lengthways.

Molybdenum

No symptoms of molybdenum deficiency appeared at any time. The plants seemed larger and healthier than controls and both tops and roots had significantly greater dry weights than the latter. The reason for this is difficult to find. It is now known that the purification methods used are not effective in removing molybdenum completely, so that enough may have been left as an impurity to supply the needs of the plants. It is also conceivable that if this were so, plants given molybdenum, even the supposedly minute amounts used, received enough to be toxic.

Whatever the explanation, the point needs further investigation. It was unfortunate that in the present investigation there were only three plants instead of five in this group, so that final yields were more heavily weighted by one particularly large plant than they might otherwise have been.

Boron

Symptoms of boron deficiency could hardly be expected to appear, since pyrex glass was used throughout the investigation. Several seedlings showed early symptoms of it in that their secondary roots were short and stumpy, so it was decided to continue one set of plants without boron. Apart from these early symptoms, from which the plants soon recovered, there was, however, little difference in appearance between control and "boron deficient" plants, the latter if anything looking rather better. Dry matter yields of roots of plants given no boron were greater than those of control plants, though the difference was not significant.

The effect of boron deficiency in timothy also requires further investigation with stricter control. While it is unlikely that this element is unessential for timothy, the plants in this investigation were presumably able to obtain enough from the glass culture

vessels and the glass distilled water to meet their requirements.

Iron

No deliberate attempt was made to induce iron deficiency, since its symptoms were already known from previous experiments. On several occasions in the early part of the experiment, however, indications of iron deficiency appeared in many of the plants. The typical symptom is severe chlorosis of the youngest leaves, any emerging after the first appearance of it being almost completely colourless. This condition usually appeared one or two days before plants were due for a dose of iron, and if this was given immediately the symptoms were noticed, recovery took place in two or three days.

A point of interest is that iron deficiency symptoms never appeared in plants deficient in copper, zinc or manganese, even if the plants were the same size as those in other treatments in which they did appear. This might be explained in zinc deficient plants by reference to their reduction in growth and consequent smaller iron requirement than that of normal plants. In manganese deficient plants it can be explained by the low manganese content, since it is now generally agreed that both manganese and iron deficiencies are induced more by lack of balance between these two elements than by the absolute level of either.

Absence of iron deficiency in copper deficient plants is, however, more difficult to explain. Their rate of growth was apparently not affected until after severe copper deficiency symptoms appeared, so the explanation offered in the case of zinc deficiency can not be applied. That applied to manganese deficient plants might. Wallace and Hewitt[3]) state that iron appears to be immobilised in the plant by toxic doses of zinc, also that copper and cobalt appear antagonistic to iron and manganese. Hewitt[6]) has since shown that iron deficiency symptoms can be induced by application of several other heavy metals, including copper, zinc, manganese and molybdenum. His work suggests that toxic effects of these heavy metals may be due in part to the disturbance they cause in iron metabolism. In a previous (unpublished) experiment the author found that too high a level of copper caused lucerne (*Medicago sativa*) plants to die apparently from iron deficiency. Possibly the iron in copper deficient plants is more efficient than that in plants adequately supplied with copper; whether it is immobilised by other

heavy metals is questionable, since it is usually considered a non-mobile element in any case (Müller[8]).

It seems logical to conclude that if too high a level of copper, zinc or manganese at a certain level of iron caused iron deficiency symptoms to appear, plants not supplied with one or more of these elements will not develop iron deficiency so readily as those which are so supplied. The whole question of iron metabolism is complicated, however, and requires considerably more investigation.

From a practical point of view, it would seem advisable to use larger amounts of iron for timothy than were used in this experiment; probably the addition of the same amount every other day instead of twice a week would be sufficient.

CONCLUSIONS

The results here described show that copper, manganese and zinc are all essential for healthy growth of timothy. Furthermore, the effects on growth and the characteristic symptoms caused by deficiencies of these elements are similar to those previously described by other workers for different species.

Proof of the necessity of boron and molybdenum has not been obtained, but it seems fairly certain that the techniques used were insufficient to exclude these elements, so no grounds appear for suggesting that either of them is not essential.

Various functions have been proposed for these elements, but it is not within the scope of this paper to discuss them, particularly as the results described throw no further light on this aspect of the subject.

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SUMMARY

An experiment is described in which the effects were studied of deficiencies of boron, copper, manganese, molybdenum and zinc on timothy grown in solution culture.

Characteristic symptoms of copper, manganese and zinc deficiencies developed and are described.

No symptoms of boron or molybdenum deficiencies appeared; plants given no molybdenum gave bigger dry matter yields than controls.

Iron deficiency appeared fortuitously in some treatments but not in others.

These results are discussed with reference to previously published work on similar lines.

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RÉPARTITION ET VARIATIONS DU SYSTÈME ASCORBIQUE CHEZ LES VÉGÉTAUX

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INTRODUCTION

Principalement étudié sous l'aspect de vitamine en physiologie humaine et animale, l'acide ascorbique a un rôle moins bien connu en physiologie végétale.

En vue de préciser ce rôle, le présent travail a étudié systématiquement la répartition et les variations de l'acide ascorbique dans les divers organes des plantes. Corps essentiellement réducteur, qui

se transforme en acide déhydroascorbique par une réaction réversible, il peut difficilement être séparé de ce dernier; aussi avons-nous étudié parallèlement la répartition et les variations de l'acide déhydroascorbique. La mesure du rapport entre ces deux formes, que nous avons désignée par l'expression „Quotient d'Oxydation réversible [11]) nous a aidé à comprendre certains aspects du rôle oxydo réducteur de ce système.

§ 1. *Le système oxydo réducteur ascorbique.*

Par la perte des deux hydrogènes de son groupement diénolique, l'acide ascorbique se transforme en acide déhydroascorbique, et par la perte d'un seul H, il prend la forme semi-oxydée ou forme S.

Cette faculté de perdre un ou deux H fait de l'acide ascorbique un réducteur énergique, cependant que l'acide déhydroascorbique qui regagne non moins facilement les deux hydrogènes est un oxydant d'une égale énergie. Ces deux corps forment un système oxydo réducteur dont le potentiel ou rH est très sensible à divers facteurs: pH du milieu, lumière, température. Or un système oxydo réducteur réduit les systèmes de rH plus élevé que le sien, et oxyde ceux de rH inférieur. Cette loi permet de situer les transporteurs (ou transmetteurs) d'hydrogène soit dans la chaîne de dégradation des corps qu'ils permettent d'oxyder, soit dans la chaîne anabolique de construction des corps qu'ils permettent de réduire. Mais il suffit que les conditions du milieu changent pour que change aussi le sens de l'action du système qui d'oxydant peut devenir réducteur ou inversement.

Il apparait donc qu'un même corps peut être inhibiteur ou activeur d'oxydation, donateur ou accepteur d'hydrogène vis à vis du système ascorbique, suivant les circonstances. Il ne faut pas oublier, l'action des diastases dont beaucoup nous échappent encore totalement ou partiellement, mais qui dans la cellule vivante doivent avoir une importance primordiale: si dans tel ou tel groupe de cellules vivantes l'acide ascorbique oxyde ou est oxydé par un corps donné, nous avons tout lieu de penser qu'il le fait sous l'action d'une enzyme dont l'action est elle-même conditionnée par le milieu physico chimique intra cellulaire au sein duquel elle agit.

§ 2. *Dosage de l'acide ascorbique.*

L'action réductrice de l'acide ascorbique a été mise à profit pour son dosage

par divers auteurs, entre autres par Tillmans, employant le 2–6 dichlorophénol-indophénol, méthode simplifiée par Sosa[25]) et par Martini et Bonsignore[16]), employant le bleu de méthylène, méthode ultérieurement modifiée par Mentzer[17]) et considérée comme plus spécifique, mais moins rapide que la première.

Nous avons généralement employé la méthode à l'indophénol pour nos premiers dosages, et celle au bleu de méthylène pour nos vérifications.

§ 3. *Dosage de l'acide déhydroascorbique.*

Nous rappellerons brièvement ici la technique de nos dosages, déjà indiquée au bulletin de la Société Botanique de France[12]), et qui comprend les opérations essentielles suivantes: broyage de l'échantillon en présence d'un volume déterminé d'acide métaphosphorique en solution aqueuse à 5%.

Première défécation et filtration. — Le filtrat est séparé en deux parties: une partie sert au dosage de l'acide ascorbique après une 2ème défécation effectuée par saturation au sulfate neutre d'ammonium, et neutralisation de l'acidité par le carbonate de calcium. L'autre partie est portée à pH = 6,2 par le carbonate de Ca, puis traitée par un courant de SH_2 passant bulle à bulle pendant 10 à 15 minutes. Elle est ensuite saturée de sulfate d'ammonium, puis débarrassée de SH_2 par un léger courant de Co_2 ou d'azote. Toutes ces opérations sont effectuées le plus possible à l'obscurité et à la température de la glace fondante pour éviter ou réduire à des quantités négligeables toutes pertes d'acide ascorbique par oxydation. Cette partie est prête pour le dosage de l'acide total (ascorbique + déhydroascorbique) qui est effectué comme celui de l'acide ascorbique seul par l'une des méthodes précédemment indiquée. L'acide déhydroascorbique est donné par la différence des deux dosages.

Cette méthode ne permet pas de faire la différence entre l'acide totalement oxydé ou déhydroascorbique et l'acide semioxydé ou forme S. Il en résulte que les quantités X trouvées d'acide déhydroascorbique, sont peut-être, en réalité, des quantités 2X de forme S, ou un mélange des deux formes. Notre quotient d'oxydation réversible est moins un rapport:

$$\frac{\text{„acide déhydroascorbique"}}{\text{acide ascorbique}}$$

qu'un rapport:

$$\frac{\Sigma \text{ des formes oxydées}}{\Sigma \text{ des formes réduites}}.$$

Nous n'avons jamais trouvé trace „d'acide combiné".

Dans les pages suivantes, la valeur du quotient d'oxydation réversible sera souvent exprimée par la fraction simple la plus approchée du rapport réel des chiffres donnés par l'analyse.

Chapitre I

Le système ascorbique dans les feuilles

Données antérieurement acquises.

Des études des nombreux auteurs qui se sont penchés avant nous sur cette question, un certain nombre de points sont fermement acquis: la feuille est, d'une manière générale une des parties les plus riches de la plante en acide ascorbique; cependant quelques fruits, dont le piment, quelques fleurs dont le *Citrus trifoliata*, sont actuellement connus pour réaliser dans leurs tissus des concentrations en vitamine C supérieures à celles des tissus foliaires.

Mais le taux de vitamine C de la feuille est variable avec divers facteurs, parmi lequels nous distinguerons: 1°) l'état de développement et l'état fonctionnel: âge de la feuille, position sur la tige, âge du végétal, forme générale ou fonctionnelle de la feuille, sa pigmentation.

2°) les tendances héréditaires, des différentes espèces.

3°) les facteurs du milieu extérieur: lumière, température, milieu écologique et nutritif.

Nous verrons avec l'étude des facteurs écologiques, l'influence marquante de la lumière et de la température, comme de l'heure de la journée, sur le taux d'acide ascorbique des feuilles. Aussi indiquerons nous pour certains dosages, les conditions météorologiques de la cueillette et des 24 heures qui l'ont précédée. Le taux moyen d'acide ascorbique dans la feuille est, de plus, un caractère variétal excessivement changeant, puisqu'il peut aller du simple au décuple suivant les diverses variétés d'une même espèce.

§ 1. *Influence de la morphologie de la feuille:*

a) Limbe et pétiole. Si dans l'étude des facteurs de variation nous commençons par celui-là, c'est que nous entendons faire immédiatement la différence entre les tissus conducteurs et les tissus assimilateurs, donc, par voie de conséquence, entre le limbe et le pétiole d'une feuille normale; en effet, le taux d'acide ascorbique du pétiole ne dépasse pas, en général, la moitié de celui du limbe qui lui correspond (tissus frais en pleine turgescence). Ainsi pour un limbe de feuille de Cassis (*Ribes nigrum* L.) contenant en moyenne 230 mg d'acide ascorbique pour 100 g de tissus frais, le

pétiole contient 110 mg. Chez le Ricin de Zanzibar (*Ricinus zanzibariensis* L.) pour un taux moyen du limbe de 190 mg. le pétiole accuse un taux de 70 mg. Chez le Persil, (*Petroselinum sativum* Joffm.) pour une moyenne des limbes des folioles de 300 mg% le pétiole dose 140 mg%. Chez *Helianthus annuus* L., 80 mg% chez le limbe, 20 mg% chez le pétiole. Chez le Tulipier de Virginie (*Lyriodendron tulipifera* L.) limbe 37 mg% — pétiole 10 mg%. Chez divers *Arum*, ces proportions sont les suivantes: *Arum maculatum* L.: limbe 125 mg% — pétiole 37 mg%. *Arum dracunculus:* limbe 179 mg% — pétiole, 67 mg%. *Richardia oethiopica:* limbe 49 mg% — pétiole 3,7 mg%. Bien tranchée en ce qui concerne l'acide ascorbique, la différence qui sépare tissus assimilateurs et tissus conducteurs ne l'est pas moins, en ce qui concerne soit le taux absolu d'acide déhydroascorbique, soit sa proportion relative par rapport au taux d'acide réduit, c'est à dire „le quotient d'oxydation" réversible de l'acide ascorbique.

Le pétiole se distingue du limbe par un quotient d'oxydation réversible très supérieur. En effet, reprenons *Ribes nigrum*: tandis que ce quotient est de 30 230 1 8 pour le limbe, il devient 35/110 ≤ 1,3 pour le pétiole. Chez *Ricinus zanzibariensis*: limbe 60 190 ≤ 1 3 pétiole 65 70 < 1. Chez *Richardia oethiopica*: limbe 18/49 < 2 5 pétiole: 26 3,7 < 8. Chez le persil (*Petroselinum sativum* L.): limbe des folioles 30 300 1 10; pétiole: 45 140 < 1/3. Chez la Clématite, (*Clematis vitalba* L.): limbe des folioles 35 317 < 1 9 pétiole: 70 70 = 1.

D'après ces quelques exemples, pris au hasard dans le règne végétal et qui vraisemblablement représentent la majorité des végétaux supérieurs de notre flore, nous pouvons retenir que: Le pétiole est sensiblement moins riche que le limbe en acide ascorbique réduit mais, par contre, il le dépasse largement, soit en valeur absolue, soit au moins en valeur relative des deux formes, par le taux d'acide déhydroascorbique.

b) Modifications fonctionnelles de la feuille. Si maintenant nous considérons une feuille profondément modifiée, par sa fonction, par exemple les feuilles réduites à des vrilles comme celles de pois, de bryone ou de citrouille, nous trouvons des modifications notables dans le taux des deux formes ascorbiques. Soit, par exemple *Bryonia dioïca* ♀ pris le 5 Juin par une journée pluvieuse au moment de la floraison: vrilles, feuilles et tiges analysées ont été

prélevées à l'extrémité d'un rameau sur les 40 derniers centimètres de celui-ci et tous ces éléments sont en pleine croissance — les vrilles ne sont pas encore enroulées autour d'un quelconque support, les feuilles ont de 1 cm, 5 à 4 cm de large.

Les chiffres trouvés sont les suivants:

Organes	Poids de l'organe		Matière sèche %	En mg% du poids frais		Quot. d'ox. rév.
	frais	sec		Ac. asc.	Ac. déhydroasc.	
Limbes . . .	1,200	0,1675	14	24	0	0
Pétioles et tiges	3,600	0.279	7,75	29	2	$\geqslant 1/15$
Vrilles. . . .	0,550	0,051	11,0	96	9	$\leqslant 1/10$

Les vrilles de Bryone ont donc une richesse en acide ascorbique de beaucoup supérieure à celle des limbes des feuilles correspondantes et un quotient d'oxydation réversible qui les rapproche plus des tiges que des limbes.

Chez *Cucurbita maxima* L. (var. Potiron jaune de Paris) les données sont les suivantes:

Organes	Poids de l'organe		% de mat. sèche	En mg% de poids frais		Quot. d'ox. rév.
	Frais	Sec		Ac. asc.	Ac. déhyd.	
Limbes . .	2,730 g	0,570 g	20,8	60	15	1/4
Pétioles . .	3,290 g	0,365	11,1	21	5,5	1,4
Tige	3,252 g	0,314	9,6	11	2,0	1/5
Vrilles non enr. . . .	1,007 g	0,0985	9,8	27	7,5	1/4
Vrilles enroulées . . .	1,012 g	0,100	9,9	11,6	0	0

Enfin chez *Pisum sativum* L. (var. Senateur), les quotients d'oxydation réversibles sont (en mg% de substance fraîche):

pour les stipules: 45/145 soit un rapport . . . . . $\leqslant 1/3$
pour les folioles . . . . . 35/178 ,, ,, ,, $\leqslant 1/5$
pour les vrilles (non enroulées) . . . 21/25 ,, ,, ,, $\leqslant 1$
pour les tiges . . . . . 15/20 ,, ,, ,, $= 3/4$

Les vrilles surtout formées de tissus conducteurs et de soutien se rapprochent donc très fortement des organes conducteurs de la plante (tiges et pétioles) avec une richesse en vitamine C qui n'est peut être pas étrangère au travail qu'elles doivent normalement fournir pour fixer la plante sur un support.

c) Forme ordinaire de la feuille. L'exemple des folioles de pois nous ammène à parler des feuilles composées et à voir comment se répartit la vitamine C dans les différentes folioles: leur teneur est loin d'être égale pour toutes, peu importe d'ailleurs qu'il s'agisse de feuilles composées pennées ou de composées palmées, les variations du taux d'acide ascorbique sont du même ordre dans ces deux sortes de feuilles: minimum pour les folioles les plus voisines du rameau, il augmente régulièrement jusqu'à la foliole terminale chez les pennées, jusqu'à la folioles opposée au pétiole chez les palmées.

Ainsi chez *Clematis vitalba* .	folioles du bas de la feuille:	51 mg%
des tissus frais	„ intermédiaires	59 mg%
„ „ „	„ terminales	94 mg%

Chez le lupin vivace à fleurs bleues:

Les deux folioles inférieures	120 mg% de tissus frais
„ „ „ suivantes	130 „ „ „ „
„ „ „ „	150 „ „ „ „
la foliole terminale.	190 „ „ „ „

Le quotient d'oxydation réversible diminue de la base au sommet, mais pas toujours de façon constante ou régulière; nos quelques observations faites sur ce sujet ne sont pas concordantes:

Ainsi chez *Phaseolus vulgaris* L. (var. noir de Belgique):

les deux folioles de la base	31/157 = 1/5
la foliole terminale	40/180 = 1/4,5

Chez *Solanum tuberosum* L. (var. Esterlingeen):

les folioles de la base	2/25
„ „ intermédiaires (indosées)	
la foliole terminale	2/30

Le comportement des feuilles simples est d'ailleurs identique. Voici les valeurs trouvées sur une feuille de *Ricinus zanzibariensis*, divisées en secteurs par sa forme et ses nervures — chaque secteur traversé au milieu par sa nervure principale pouvant être assimilé à une foliole de feuille composée:

secteur No. 1 (base)	36/175 mg%
secteur No. 2	38/198 mg%
secteur No. 3	37/207 mg%
secteur No. 4 (sommet)	39/210 mg%

§ 2. *Influence de l'âge de la feuille et de sa position sur la tige.* Au cours d'une précédente étude[13]), nous avons montré par l'examen détaillé du système foliaire de *Ribes nigrum* et d'*Hélianthus annuus*, que chez les Dicotylédones l'influence de l'âge de la feuille se double de celle d'un autre phénomène: celui du changement de position de la feuille sur la tige au fur et à mesure que celle-ci s'allonge et pousse de nouvelles feuilles. Non seulement la feuille cesse de se développer et vieillit, mais elle passe de première position tout au sommet de la tige, à la seconde, troisième, puis ennième position, s'éloignant ainsi de plus en plus de la zône active de croissance de la tige et des feuilles. Chez *Ribes nigrum*, le maximum d'acide ascorbique se situe dans les premières feuilles du haut de la tige en voie de croissance — souvent dans la 3ème feuille où il correspond à un minimum d'acide déhydroascorbique; le maximum de celui-ci se rencontre immédiatement au-dessus dans les toutes premières feuilles en voie d'épanouissement.

Dans le temps, on note 2 maxima pour les 4 premières feuilles: l'un au départ de la végétation au début d'Avril; l'autre courant Juin.

Chez *Helianthus* A., la plus forte teneur en acide ascorbique se rencontre dans les 2 premières feuilles tout au long de la végétation, avec un maximum avant la floraison du capitule principal. La forme oxydée se rencontre également dans les premières feuilles et avant la floraison.

Voici maintenant le cas de deux autres Composées: l'Artichaut et le Chrysanthème horticole:

Cas des plantes vivaces. Chez l'artichaut (*Cynara scolymus*) pris le 15 Juin avant la floraison, les feuilles jeunes encore blanchâtres et repliées sur elles mêmes — et les feuilles plus âgées bien vertes et bien étalées, les taux respectifs sont les suivants:

	Ac. ascor.	Ac. déhydro.	Total	Quotient d'ox. rév.
Feuilles jeunes	22 mg%	15 mg%	37	2/3
,, adultes	72 mg%	24 mg%	96	1/3

Il y a donc une augmentation très appréciable du taux d'acide ascorbique et déhydroascorbique, mais baisse non moins sensible du quotient d'oxydation réversible qui retombe de 2/3 à 1/3.

Nous ne quitterons pas la famille des Composées sans examiner

une plante, le Chrysanthème, var. horticole à fleurs rouges, qui par sa floraison préhivernale, est susceptible de nous donner des indications sur l'influence de l'arrière saison sur la répartition de la vitamine C chez ses divers étages de feuilles (par la méthode à l'indophénol):

	Ac. ascorb.	Ac. déhydro.	Ac. total	Quotient d'ox. rév.
Feuille No. 1				
Dessous capit	67,5	44,5	112	2/3
Feuille No. 2	84	56	140	2/3
Feuille No. 3	80	80	160	1
Feuille No. 4	67	67	134	1
Feuille No. 5	85	42	127	1/2
Feuille No. 6	70	18	88	1/4

Chez cette plante qui se distingue de toutes celles examinées jusqu'à maintenant par sa très forte teneur en produits réducteurs après passage de l'hydrogène sulfuré dans la préparation, donc assimilables à l'acide déhydroascorbique, le maximum d'acide ascorbique est atteint par la 2ème feuille: et le maximum de déhydroascorbique par la 3ème feuille. Le quotient d'oxydation réversible est maximum pour les deux feuilles No. 3 et 4. Cette plante d'arrière saison ne diffère donc pas de la plante de pleine saison (*Hélianthus annuus*) pour la répartition du taux d'acide ascorbique. Elle s'en écarte légèrement par une distribution plus régulière de l'acide déhydroascorbique — peut être faut-il voir là l'effet d'une circulation moins intense de la sève et d'un transport moins actif des produits élaborés.

Cas d'une monocotylédone: Quittons les dicotylédones pour étudier une monocotylédone: *l'Iris hybride* des jardins (var. Dorothée). Les dosages ont été faits par la méthode à l'indophénol sur les deux tiers supérieurs de la longueur des deux plus jeunes feuilles de la plante, seuls franchement verts.

Date	État de la végétation	En mg% de matière fraiche	
		Ac. ascorb.	Ac. déhydro.
15 Janv.	au repos	258	0
11 Mars	au repos	300	0
1er Avr.	début de la croissance	492	0
15 Avr.	les fleurs sont en boutons	470	0
1er Mai	fleurs ouvertes	386	0
16 Mai	fleurs fanées	320	0
20 Août	sans signe particulier	310	0

Il ne m'a jamais été possible de mettre en évidence la moindre trace d'acide déhydroascorbique. Il nous faut donc admettre que le métabolisme de l'acide ascorbique chez cet Iris n'est pas le même que chez *Ribes nigrum* et chez *Hélianthus annuus*. Le taux maximum ne se place d'ailleurs pas au même stade végétatif: au début de la floraison chez les deux plantes précèdentes — au réveil de la végétation (1er avril) chez l'Iris Dorothée. Comment ne pas être frappé par cette différence fondamentale du groupe des plantes à quotient d'oxydation réversible positif, et d'une plante à quotient constamment nul: l'Iris précité? Chez le premièr groupe nous constatons un maximum au début de la floraison, aussi bien dans la forme réduite que dans la forme oxydée, maximum que nous savons correspondre à un sommet dans l'intensité des échanges gazeux respiratoires; tandis que chez l'Iris, s'il y a bien concordance d'un maximum d'acide ascorbique réduit avec un premier sommet des échanges gazeux respiratoires, au réveil de la végétation, il n'y a pas trace d'un second maximum au moment de la floraison. Ce fait et celui de l'absence totale de forme oxydée permettant d'affirmer que le métabolisme de l'acide ascorbique est foncièrement différent chez les plantes du type Ribes-Hélianthus et chez les plantes du type Iris. Il est permis de penser que chez ce dernier type l'acide ascorbique n'a pas de rôle dans les phénomènes oxydo-réducteurs des feuilles de ces plantes, sans quoi l'on trouverait trace de sa forme oxydee. Au contraire, chez les plantes du premier groupe, si nous rapprochons la courbe représentative des variations saisonnières des deux formes de l'acide ascorbique, de celle des autres fonctions vitales de la plante, particulièrement des courbes représentatives des fonctions respiratoires (absorption d'oxygène) et d'assimilation chlorophyllienne (absorption du gaz carbonique) nous constatons une similitude suffisante pour qu'elle nous amène à penser que l'acide ascorbique a bien, chez ces plantes, un rôle actif de substance oxydo-réductrice dont l'activité se traduit par le quotient d'oxydation réversible.

L'hypothèse d'une migration des acides ascorbiques chez *Ribes Nigrum* dans le bois au moment de la chute des feuilles, est infirmée par le fait que le bois n'est pas plus riche en acide ascorbique sur l'arbrisseau feuillu ou l'arbrisseau dépouillé:

rameau au moment de son aoûtement	37/60 mg%
rameau après la chute des feuilles	35/55 mg%

§ 3. *Influence de la pigmentation de la feuille.*

Nous savons que dans la feuille jaune ou jaunissante, l'acide oxydé a disparu et l'acide réduit est en voie de disparition. La disparition de la chlorophylle au profit de la xanthophylle, fait partie des phénomènes de sénescence sur lesquels nous ne reviendrons pas. Tout différents dans leur processus d'apparition et leur conséquences sont les pigments anthocyaniques qui colorent les feuilles jeunes de certaines variétés ou les feuilles adultes de quelques autres généralement dénommées ,,rubra" ou ,,atro purpurea". Des auteurs, parmi lesquels Huszak[10]) ont pensé que les colorations brunes, rouges ou violettes qui caractèrisent les organes d'un grand nombre de plantes sont dûes à une réduction des flavones par l'acide ascorbique avec formation de déhydroascorbique. Combes[15]) et Willstätter[28]) ont en effet montré que l'on pouvait obtenir ,,in vitro" les anthocyanes par réduction des flavones au moyen d'hydrogène naissant. ,,In vivo" il est séduisant d'admettre que cette réduction s'opère par l'acide ascorbique qui céderait son hydrogène aux flavones, lesquelles donneraient ainsi naissance aux anthocyanes, tandis que lui-même serait oxydé en déhydrosascorbique. La conséquence la plus immédiate d'une telle hypothèse est que les organes colorés par les pigments anthocyaniques doivent être particulièrement riches en acide ascorbique et surtout en déhydroascorbique.

C'est dans cette hypothèse que nous avons examiné quelques feuilles fortement colorées en rouge:

Iresine herbstii, var. *acuminata* { Amaranthacées). Ac. asc. 8 mg%
Amaranthacées). Ac. dehyd. 5,5 mg%

Si la proportion de forme oxydée est relativement forte par rapport à la forme réduite, le taux absolu des deux formes est dérisoire dans une feuille aussi violamment colorée que celle de cette Amaranthacée. Il est vrai que les dosages ont été faits fin Octobre, mais la plante était encore en pleine végétation.

Chez *Beta vulgaris*, var. rouge longue potagère, les feuilles entièrement pourpres contenaient fin Octobre également:

Ac. ascorb. 52,50 mg% — Ac. Déhydro. 10.30 mg% — Quot. d'O.R 1,5 tandis que les feuilles d'une betterave abâtardie dont les nervures seules étaient colorées en rouge dosaient. . Ac. asc. 41,40 mg%. Ac. déhyd. néant.

S'il y a là une indication en faveur de l'hypothèse formulée plus haut, il faut remarquer que: 1°) dans les feuilles vertes, dépourvues totalement de forme oxydée, les anthocyanes n'étaient pas totalement absents, puisque les nervures étaient colorées.

2°) les facteurs génétiques différents des deux individus étudiés peuvent fort bien être responsables de la différence des taux d'acide ascorbique.

Les Cupuliféracées nous ont fourni deux autres sujets d'étude avec *Fagus sylvatica* et sa variété atropurpurea et *Corylus avellana* et sa variété rouge. Nous avons trouvé en Juillet, pour les limbes du hêtre à feuilles vertes: (40,6% de matière sèche):

Ac. ascorb. 147 mg% – Ac. déhydro. 24 mg% – Quot. d'ox. rév. 1/6

du hêtre pourpre: (44,44% de matière sèche):

Ac. ascorb. 149 mg% – Ac. déhydro. 24 mg% – Quot. d'ox. rév. 1/6

du noisetier à feuilles vertes (33,80% de matière sèche):

Ac. ascorb. 149 mgr% – Ac. déhydro. 24 mg% – Quot. d'ox. rév. 1/6

du noisetier rouge (27,90% de matière sèche):

Ac. ascorb. 172 mg% – Ac. déhydro. 13 mg% — Quot d'ox. rév. 1/13

Les indications de ces deux espèces de Cupuliféracées sont donc assez nettement contraires à l'hypothèse d'H u s z a k — mais on peut encore reprocher à ces deux observations de n'avoir pas tenu compte des facteurs génétiques forcément différents entre la variété commune et la variété pourpre. Aussi, pour éliminer l'influence possible d'un génômes différent, avons nous pris des feuilles vertes et des feuilles rouges sur un même pied de *Berberis vulgaris* — feuilles de mêmes dimensions, mais les vertes un peu plus jeunes que les rouges ainsi que l'indique le pourcentage de matière sèche:

	Pds frais	Pds sec	Matière seche %	En mg% mat. fraiche		Quot. d'ox. rév.
				Ac. asc.	Ac. déhydr.	
feuilles rouges	0,380	0,1335	35.13	515	255	1/2
feuilles vertes	0,410	0,1325	32,31	650	475	2/3

Malgré la légère différence d'âge entre les deux sortes de feuilles, cette observation, jointe aux précédentes, semble bien trancher le débat dans un sens négatif. Cependant, nous avons fréquemment observé au cours de nos dosages qu'une légère teinte rose de nos extraits métaphosphoriques correspondait souvent à un quotient

d'oxydation réversible élevé. Si l'acide ascorbique n'est pas nécessaire à la formation des anthocyanes, par contre on peut penser que chez certains végétaux, les flavones sont nécessaires à l'oxydation de l'acide ascorbique.

§ 4. *Variation du taux d'acide ascorbique et du quotient d'oxydation réversible avec les espèces.*

C'est un point particulier du problème plus général des rapports de la systématique avec la physiologie. Si cette dernière a souvent ratifié les arrêts de la classification morphologique en confirmant l'identité des mécanismes physiologiques de deux variétés ou même de deux genres voisins, elle a parfois aussi fait apparaître des divergences fondamentales entre des individus qui semblaient par leurs formes extérieures extrêmement proches l'un de l'autre: c'est le cas de nos variétés de plantes de grande culture dont les unes résistent au froid ou à la sécheresse, ou aux maladies cryptogamiques, tandis que les autres y sont sensibles. Les différences physiologiques atteignent même parfois des variétés réputées parfaitement pures, c'est-à-dire parfaitement homogènes du point de vue morphologique et, semblait-il, du point de vue génétique; et qui se séparent soudain en groupes jouissants de propriétés diverses. Au sein des variétés dites pures, les sélectionneurs ont dû admettre l'existence de races physiologiques" dans l'impossibilité où ils sont de distinguer ces races par un caractère morphologique. Nous ne devions pas nous attendre à moins de variabilité dans le taux d'une substance aussi labile que l'acide ascorbique et c'est bien en effet ce que confirme l'expérience des innombrables chercheurs qui se sont déjà penchés sur cette question. Nous n'avons voulu ici qu'apporter notre modeste pierre à l'édifice déjà considérable de la connaissance de la richesse des végétaux en acide ascorbique et à l'édifice naissant du quotient d'oxydation réversible.

Pour que les chiffres cités puissent avoir une valeur comparative il est nécessaire que l'analyse de la feuille saisisse la plante à un moment bien déterminé de son évolution annuelle. Nous nous sommes donc efforcé de faire nos dosages sur des plantes en fleurs, en prenant les feuilles du tiers supérieur de la tige. Dans le tableau ci-contre, qui groupe quelques uns de nos résultats, nous avons également indiqué le lieu et l'époque du prélèvement afin de circonscrire les conditions extérieures dont nous verrons toute l'importance

Famille	Plante	Lieu de récolte	Epoque	Etat de la végétation	En mg% de matière fraiche			Quotient d'ox. rév.
					As. ascorb.	Ac. déhydro.	Ac. total	
Renonculacées	*Ranunculus acris*	St. Cloud	Mai	floraison	73	19	92	1/4
Cruciféracées	*Cheiranthus cheiri*	St. Cloud	Avril	,,	112	27	139	
,,	*Alliaria officinalis*	,,	1er Mai	,,	156	22	178	1/7
,,	*Iberis sempervirens*	Paris	Novembre	ordinaire	283	31	314	1/9
,,	*Brassica napus oleif.*	Paris	Novembre	ordinaire	112	10	122	1/11
,,	*Brassica napus esculens*	St. Cloud	Avril	floraison	112	11	123	1/10
Papilionacées	*Sarothamnus scop.*	Paris	Fevrier	ordinaire	147	7	154	1/21
,,	*Cytisus laburnum*	St. Cloud	1er Mai	floraison	239	05	364	2/19
,,	*Lotus corniculatus*	Paris	Juillet	,,	120	11	101	1/11
,,	*Phaseolus vulgar*	St. Cloud	Septemb.	,,	110	38	140	1/4
,,	*Sosa hispida* var.	Paris	Juillet	,,	148	38	186	1/4
,,	*Trifolium pratensis*	St. Cloud	Mai	,,	168	100	268	10/17
,,	*Pisum sativum*	St. Cloud	Mai	,,	178	35	213	1/5
	Wistaria sinensis	St. Cloud	,,	,,	370	33	403	1/11
,,	*Albizzia julibrisen*	Paris	fin Août	,,	595	95	680	1/6
,,	*Acacia penninervis*	en serre	Décembre	ordinaire	61	0	61	0
,,	,, *linifolia*	,,	,,	,,	51	0	51	0
,,	,, *sentis*	,,	,,	,,	37	0	37	,,
,,	,, *calinifolia*	,,	,,	,,	50	0	50	0
Composacées	*Cynara cardonculus*	Orly	Décembre	Végét. hiv.	32	4,5	06,5	1/7
,,	,, *scolimus*	,,	Juin	floraison	72	24	96	1/3
,,	*Matricaria chamomillia*	Paris	Décembre	floraison	56	5	61	1/11
,,	*Calendula arvensis*	,,	,,	,,	28	7	35	1/4
,,	*Chrysanthemum* var. hort.	Paris	Novembre	,,	31	31	62	1/1
,,	,,	,,	Mai	ordinaire	19	26	45	1
,,	*Artemisia vulg.*	Paris	Novembre	floraison	22	22	44	1
,,	,,	,,	Mai	ordinaire	56	41	97	1
,,	*Leontodon autumnalis*	Paris	Décembre	gelée	20	5	25	1/4
,,	*Hélianthus annuus*	St. Cloud	Août	floraison	60	6	66	1/10
,,	,, *tuberosus*	,,	,,	,,	30	4	34	1/8
Solanacées	*Solanum nigrum*	Paris	Septembre	fin flor.	60	2	60	1/30
Liliacées	*Ruscus aculeatus*	Paris	Février	shivernal	140	8	148	1/17
,,	,, *hypoglossum*	,,	,,	,,	168	0	168	0
,,	,, *racemosus*	,,	,,	,,	126	0	126	0
Iridacées	*Iris hybr.* Dorot.	Paris	Mai	floraison	386	0	396	0
,,	*Gladiolus hyb.*	St. Cloud	Juillet	,,	478	19	497	1/25
Aroïdacées	*Arum dracumculus*	Paris	Août	,,	179	11	190	1/15
,,	,, *maculatum*	Angers	Mai	,,	125	indosé	—	—
,,	,, *italicum*	Paris	Avril	,,	130	13	143	1/10
,,	*Richardia oethiopica*	Paris (intérieur)	Janvier	,,	49	18	67	3,6/10

dans un prochain chapitre. Malgré l'inévitable diversité des résultats entrainée par la diversité des circonstances écologiques et du milieu externe, les familles se résolvent en un certain nombre de groupes au sein desquels les espèces ou variétés qui les composent, se caractérisent non pas par un taux déterminé d'acide ascorbique: (ainsi chez les Cruciféracées, le groupe *Cheiranthus*, *Brassica* avec 112 mg%), mais par une tendance héréditaire à produire ou à accumuler une certaine quantité de vitamine C dans leurs tissus: c'est en un mot, un caractère fluctuant comme le sont tous les caractères placés sous la dépendance du milieu externe.

Chez les Papilionacées, le groupe *Lotus-Phaseolus-Soja* et *Trifolium* qui s'échelonne de 110 à 168 — puis le groupe exotique avec *Wistaria* et *Albizzia* qui va de 370 à 595. Le genre *Acacia* est à mettre à part, étant donné les conditions très particulières de sa végétation dans une serre, en plein hiver, lorsqu'ont été faits nos dosages.

S'il est permis de parler de groupes plus ou moins homogènes en ce qui concerne la teneur des végétaux en acide ascorbique, il semble par contre tout-à-fait impossible de faire la même classification en ce qui concerne l'acide déhydroascorbique, et par voie de conséquence, le quotient d'oxydation réversible.

Est-ce à dire qu'il y ait opposition entre les deux formes ascorbiques? Nous ne le pensons pas. Leur taux dans les tissus de la plante sont tous deux des caractères fluctuants soumis aux influences extérieures. Mais l'amplitude des variations du second, beaucoup plus considérables que celles du premier, ne nous permet pas encore de grouper les plantes autour d'un taux moyen de forme oxydée. Cependant, au chimisme particulier de chaque espèce ou variété doit correspondre la faculté de former, conserver ou accumuler une quantité fixe d'acide déhydroascorbique dans des circonstances déterminées.

Nous estimons probable la concordance du groupement des espèces réalisé suivant ces données avec celui que nous avons pu établir suivant le taux de l'acide ascorbique.

Chapitre II

LE SYSTÈME ASCORBIQUE CHEZ LES PIÈCES FLORALES

Connaissances antérieurement acquises.

Résultant d'une modification morphologique de la feuille, les pétales et sépales nous offrent d'une manière générale, un exemple de

l'influence des couleurs ou de l'albinisme sur le taux d'acide ascorbique et le quotient d'oxydation réversible que nous avons déjà noté en étudiant la feuille proprement dite. Mais il s'y ajoute l'action particulière des fonctions de reproduction qui sont dévolues à l'ensemble floral. Ainsi Mme S o s a- B o u r d o u i l [21]) a montré comment varie l'acide réduit avec l'ouverture des pièces florales et la fécondation chez *Iberis sempervirens, Matthiola fenestralis, Iris germanica* et *orientalis*. Elle a mis en évidence:

1°) une chute brusque du taux d'acide ascorbique chez les étamines au moment de la maturation du pollen;

2°) l'absence à peu près complète d'acide réduit dans le pollen mûr

3°) une chute brusque dans l'ovaire de l'Iris au moment de l'ouverture des étamines, chute suivie d'un accroissement non moins brusque au moment de l'ouverture des stigmates. Ensuite, suivant que l'on considère la quantité d'acide ascorbique par ovaire ou le taux pour 100 de matière fraiche, il y a accroissement régulier dans le premier cas, ou légère dimunition du taux envisagé dans le second.

§ 5. *Les Iris.*

Etudiant divers Iris, nous avons pour notre part, trouvé les chiffres suivants, par la méthode à l'indophénol. En mg pour 100 gr de tissus frais:

Iris hybride des jardins, var. Dorothée — fleur bien épanouie — étamines ouvertes.

	Poids de d'organe	Ac. ascor.	Ac. déhydro.	Quot. d'O. R.
Sépales	0,850	257	0	0
Pétales	0,828	234	0	0
Styles et stigmates . .	0,250	143	0	0
Ovaire	0,600	218	22	1/10

d'autre part, étudiant le taux de vitamine C au cours de l'évolution de l'ovaire de ce même Iris et par la même méthode, nous avons trouvé les résultats suivants:

Stade floral	Poids de l'ovaire	Ac. ascor.	Ac. déhydro.	Quot. d'O. R.
Fleur fermée	0,408	226	42	1/5
Fleur ouverte depuis peu	0,500	179	33	1/5
Etamines ouvertes. . .	0,550	196	54	1/4
Stigmates ouverts. . .	0,600	218	22	1/10

Mais chez cet hybride, la fécondation est plus ou moins bonne; le plus souvent, le fruit avorte, la graine ne se forme pas, parce que les chromosomes du pollen et de l'ovule n'ont pu s'apparier. Aussi avons nous étudié l'ovaire de deux espèces pures et fécondes:

Iris pallida et *Iris germanica.*

Stade floral	*Iris Germanica*				*Iris Pallida*			
	Poids de l'ovaire	Ac. as.	Ac. déh.	Quot. d'ox. rév.	Poids de l'ovaire	As. as.	Ac. déh.	Q. d'ox. rév.
1) bouton fermé	0.212	353	12	1/30	0,285	262	14	1/19
2) étamines ouvertes	0,480	280	24	1/12	0,331	396	14	1/28
3) stigmates fécondés	0,751	308	11	1 30	0,362	168	62	1/3

Dans les ovaires de ces deux Iris, ou bien le chimisme ascorbique est foncièrement différent, ou bien: Chez *Pallida,* les phénomènes sont plus longs à se produire que chez *Germanica*: les stades floraux ne se correspondent pas ou se correspondent avec un certain décalage dans le temps: 2 et 3 chez *Pallida* étant l'équivalent de 1 et 2 chez *Germanica*. Pour permettre la comparaison des parties sexuées et non sexuées, nous avons fait le dosage du tronçon de la hampe florale situé juste au-dessous de la première ramification de la grappe.

	Iris Germanica (des st. 1 et 3)			*Iris Pallida* (des st. 2 et 3)		
	As. asc.	Ac. déh.	Quot. d'O. R.	As. asc.	Ac. déh.	Quot. d'ox. rév.
Hampe florale	582	22	1/26	162	8	1/20
Bractée	448	12	1/37			

Chez les Iris, il existe donc de grandes différences variétales tant au point de vue de la teneur absolue en acide ascorbique qu'au point de vue des variations de celui-ci au cours de l'évolution de la fleur et de la fécondation. En outre, tandis que les pétales et les sépales se rapprochent des feuilles par leur quotient d'oxydation réversible, nul, les bractées de la grappe forment transition entre les feuilles et les ovaires. Ceux-ci se rapprochent à leur tour des axes floraux qui les portent et qui, chez Germanica sont nettement plus riches qu'eux en acide réduit.

§ 6. *Les Arum.*

Un autre exemple des variations du taux d'acide ascorbique sous

ses deux formes en corrélation avec l'évolution florale nous est fourni par l'étude d'*Arum dracunculus* L. dont la curieuse inflorescence se prête assez bien à l'étude qui nous occupe dans chacun des ensembles fonctionnels.

Nous avons en outre cherché à déterminer s'il existait ou non une corrélation entre les variations du taux d'acide ascorbique et celles de la température de ces inflorescences: variations minutieusement décrites par Garreau en 1851, puis par Senebier, Goeffert et Brougniart et reprises par L. Blaringhem [3]) dans sa belle étude sur la fièvre des Arum où il démontre que le maximum de température qui a lieu dans la région des fleurs mâles, correspond à une quantité considérable d'oxygène consommé.

Les dosages d'acide ascorbique ont été faits par la méthode au bleu de méthylène, la mesure des températures par un couple thermo électrique monté en aiguille très fine que l'on pique au sein des tissus étudiés. Le couple est relié à un galvanomètre très sensible, gradué directement en degrés centigrades. La température du sol est prise pour zéro.

Voici comme premier exemple, une inflorescence dont la spathe n'est pas encore ouverte: l'androcée a tout son pollen:

Pièce florale	En mg% de matière fraiche			Temp. relative
	Ac. asc.	Ac. déh.	Quot. d'ox. rév.	
Spadice	133	4	1/35	—4°
Androcée	75	30	1/2,5	—3°
Partie interm. à poils glandulaires	68	24	1/3	—3°
Gynécée	53	24	1/2	—3°
Axe floral	30	2	1/15	+3°
Spathe	39	3	1/12	+1°
Bulbe	7	0	0	+1°

d'après ce tableau, il est difficile à ce stade floral de reconnaitre une corrélation quelconque entre la température et les données des trois autres colonnes. Plus intéressant est le tableau suivant concernant des fleurs plus âgées:

Pièce florale	As. asc.	Ac. déh.	Quot.d'ox.rév.	Température
Androcée avant l'ouv. des anthères	28	6	1/5	0
Androcée au moment de l'ouv. des anth.	23	10	1/2	+12°
Androcée qqs. heures après (16 h)	24	8	1/3	+2°
Partie interméd. à 16 heures	43	13	1/3	+6°
Gynécée au moment de la fécondation	28	6	1/5	+3°
Spadice même heure	89	14	1/6	+1°
Gynécée 48 h après	34	5	1/7	0
Spadice même heure	10	0	0	0

Le maximum de température +12° est atteint dans l'androcée au moment où le quotient d'oxydation réversible atteint sa valeur maxima, voisine de 1 2. Toutefois, si l'oxydation réversible de l'acide ascorbique peut avoir une part dans l'élévation de la température de cet organe, elle n'est pas seule en jeu. puisque le tableau précédent nous a montré une valeur très voisine du quotient d'oxydation réversible en l'absence de température positive. La migration intense de l'acide ascorbique du spadice vers le gynécée est d'ailleurs de nature à troubler l'observation du phénomène qui nous intéresse. A décroissance régulière du taux d'acide ascorbique que l'on observe sur l'inflorescence non épanouie, depuis l'extrémité du spadice jusqu'au bulbe, les phénomènes sexuels substituent un ordre inverse, avec minimum dans le spadice en voie de flétrissement et maximum dans le gynecée en train de développer ses fruits.

Toutefois, avant d'arriver à ce stade, 24 heures environ après l'ouverture des anthères, les taux d'acide oxydé et réduit tout le long de l'inflorescence sont les suivants:

Pièce florale	Ac. ascorb.	Ac. déhydro.	Quot. d'ox. rév.
Spadice	70	8	1/9
Androcée	24	8	1/3
Axe interméd.	30	11	1/3
Gynécée	26	13	1/3
Spathe	36	3	1/8

Avant de quitter les Monocotylédones, signalons encore les chiffres trouvés par la méthode à l'indophénol sur deux Aroïdés, prélevées dans les serres du Muséum:

	As. ascorb.	Ac. déhyd.	Quot. d'ox. rév.
1°) *Anthurium splendeni*			
parties ♂ et ♀ de l'inflorescence	40	22	1/2
Spathe	20	25	1
2°) *Philodendron giganteum*			
fleurs mâles	14	0	0
fleurs femelles	22	0	0

Ces deux inflorescences sont donc aux deux pôles de la famille des Aroïdés en ce qui concerne la valeur du quotient d'oxydation réversible: valeur nulle pour *Philodendron*, très élevée pour *Anthurium* — mais étant donné d'une part la difficulté que l'on éprouve à caractériser exactement un stade floral — et d'autre part, l'extrême variabilité des taux ascorbiques avec l'évolution de la fleur les chiffres précédents peuvent n'être que l'expression d'un stade floral différent.

Conclusions.

Chaque espèce végétale a un type de variation qui lui est propre, mais dans lequel on retrouve les traits essentiels suivants:

1°) décroissance régulière de l'acide ascorbique dans les pétales et les sépales pétalloïdes, depuis la formation du bouton, jusqu'au flétrissement final.

2°) décroissance chez les étamines, avec chute plus ou moins accentuée au moment de l'ouverture des anthères, cependant que la forme oxydée augmente.

3°) deux maxima de la forme réduite chez l'ovaire accompagnant ou suivant de près deux maxima de la forme oxydée et du quotient d'oxydation réversible. Ces variations semblent être en rapport assez étroit avec les variations de l'intensité respiratoire des diverses pièces florales.

Chapitre III

LE SYSTÈME ASCORBIQUE CHEZ LES FRUITS

En raison de leur valeur alimentaire, les fruits ont donné lieu à de très nombreuses recherches.

§ 7. *Les fruits sucrés.*

Un de ceux qui a été le plus universellement étudié est la tomate sur laquelle se sont penchés plusieurs auteurs.

Tous sont arrivés à des conclusions identiques à savoir:

que la teneur du fruit en acide ascorbique augmente régulièrement du fruit vert et dur au fruit rouge et mûr,

dans un même fruit, irrégulièrement mûr et pigmenté, ce sont les parties les plus rouges qui sont les plus riches en vitamine C.

D'après Wokes et Organ[29]) c'est l'épiderme du fruit qui serait le mieux pourvu, aussi bien dans le fruit mûr que dans le fruit vert. Ensuite, d'après Piegai et Delindati[21]), viendrait le liquide mucilagineux dans lequel baigne les graines, de 26 à 132 mg pour 100; puis la pulpe solide, de 22 à 23 mg%; enfin les graines qui ne contiennent plus que des traces d'acide réduit.

Les variations du taux d'acide ascorbique de la tomate sont donc maintenant bien étudiées mais celles de la forme oxydée le sont beaucoup moins. Aussi avons nous sommairement essayé d'y remédier car, outre l'intérêt général que nous avons attaché à la connaissance de l'évolution concomittante des deux formes, nous nous sommes posé la question de savoir si l'on peut établir une corrélation entre le changement de pigmentation du fruit

au moment de sa maturation et les variations des deux formes, une absence totale de corrélation ayant été notre conclusion vis-à-vis du système foliaire.

a) Le système ascorbique et les changements de pigmentation du fruit.

Le rougissement de la tomate a été expliqué par une altération de la chlorophylle, par les enzymes oxydants (Willstätter) avec formation de lycopène et de lycopénoïdes. Ce phénomène ne se produirait pas seulement dans le fruit mûrissant, mais également dans le fruit jeune et complètement vert: mais, tandis que chez ce dernier, une peroxydase détruit le lycopène au fur et à mesure de sa formation, dans le fruit mûrissant, une antioxydase permet l'accumulation des pigments rouges. Ceux ci résulteraient donc d'une oxydation freinée à un certain stade. Or nos recherches nous ont montré: que l'acide déhydroascorbique existait normalement chez deux variétés de tomates et qu'il y avait une tendance à l'augmentation du quotient d'oxydation réversible à partir du début du rougissement du fruit:

		Ac. ascorb.	Ac. déhydro.	Q. d'ox. rév.
Tomate „Rouge grosse lisse"	verte	21	15	3/4
	route	18	14	7/9
Cerise rouge	verte	13,3	3,8	1/4
	rouge	23,2	6,4	1/4

Mais ces chiffres, outre qu'ils sont peu probants par eux-mêmes, le sont encore moins si l'on considère qu'ils ont été obtenus sur des fruits différents, n'appartenant pas à la même grappe (cerise rouge) ou qui plus est, récoltés à des dates différentes (rouge grosse) entre lesquelles des changements de température et d'éclairement se sont produits. Beaucoup plus significatifs sont les chiffres obtenus sur un fruit voisin de la tomate, celui du piment doux d'Espagne, où les deux teintes existant sur un même fruit, nous ont permis une comparison plus exacte des teneurs en vitamine C de ces deux zones.

Piment doux d'Espagne		As. ascorb.	Ac. déhydro.	Mat. Sèche
Région du pédoncule	péricarpe vert	140 mg%	0 mg%	11%
	„ rouge	186 „	10 „	11%
Région de la pointe	„ vert	186 „	0 „	11%
	„ rouge	207 „	22 „	11%

Nous constatons donc:

1°) un enrichissement du péricarpe en vitamine C depuis la base du fruit (région voisine du pédoncule) jusqu'à la pointe.

2°) un acroissement très net de l'acide réduit dans les zones rouges

3°) l'apparition de la forme oxydée dans les zones rouges alors

qu'elle était absente des zones chlorophylliennes. Mais le fruit ci-dessus analysé, avait rougi à la lumière et température du laboratoire, séparé de son pied-mère — le pédoncule trempant dans l'eau de source. Il fallait s'assurer que ces conditions artificielles n'avaient pas d'influence pertunbatrice sur le taux de vitamine C. C'est ce que nous avons fait en dosant, huit jours auparavant, au moment de la mise en route de l'essai de maturation, un fruit complètement vert, analogue à celui qui allait mûrir. L'absence de forme oxydée et des doses de forme réduite, tout à fait voisines de celles trouvées ci-dessus dans les régions vertes, nous permettent de penser que les conditions de conservation et de maturation artificielles n'ont pas eu d'influence pertunbatrice sur le phénomène étudié.

Un autre genre de Solanées, voisin des deux précèdents, *Solanum pseudo-capsicum*, nous a également fourni un exemple de l'augmentation des deux formes ascorbiques dans le fruit rouge:

		As. ascorb.	Ac. déhydro.
Solanum pseudo-capsicum	fruit vert . . .	19 mg%	2 mg%
	,, jaune. . .	22 ,,	3 ,,
	,, rouge. . .	26 ,,	6 ,,

Barker et Parkinson[32]) étudiant à maturité un certain nombre de variétés de tomates, y ont trouvé des doses variables mais très notables d'acide déhydroascorbique.

L'étude de la pulpe du fruit de *Rosa canina*, nous apporte un aspect nouveau de la question, qui nous a manqué jusqu'ici; peut-être parceque l'étude des autres fruits n'a pas été poussée jusqu'à son terme extrême, et qui est celui-ci:la disparition totale de la forme oxydée dans le fruit complètement rouge; mais encore ferme et dur, et la diminution de la forme réduite dans le fruit parfaitement mûr où l'amidon a fait place à des sucres. Notons, en passant l'extraordinaire richesse de ces fruits d'églantiers, déjà signalée par T. Sabalitschka et H. Michels[23]).

Mais voyons plutôt les chiffres — par la méthode au bleu de méthylène.

	Pois frais	% de mat. sèche	Ac. ascor.	Ac. déhyd.	Quot. d'ox. rév.
Fruit parfait. vert . . .	0,150 g	36,30	1736 mg%	82 mg%	1/21
,, jaune rougis. . .	0,155 g	36,80	1900 mg%	296 mg%	1/6
,, parfait. rouge et dur	0,342 g	38,20	1956 mg%	0	0
,, parfait/mur, rouge et mou	0,344 g	46,00	1260 mg%	0	0

L'oxydation réversible est donc maxima dans le fruit jaune rougissant, mais devient nulle dans le fruit complètement rouge où la forme réduite est à son maximum; il serait donc nécessaire de faire l'analyse chromatographique rigoureuse de chacun des stades étudies, afin de déterminer aussi exactement que possible la transformation pigmentaire qui correspond à cette oxydation; cette transformation semblant à priori, se situer à la hauteur des pigments xanthophyliens-caroténiens, beaucoup plus que dans la formation des pigments anthocyaniques, ce qui expliquerait l'absence de corrélation constatée à propos des feuilles. Il est vrai que les circonstances atmosphériques particulières du milieu de Décembre, époque à laquelle ont été faits ces dosages, peuvent très bien troubler l'observation exacte des phénomènes oxydatifs en favorisant par le froid, l'accumulation de quantités importantes d'acide ascorbique.

Peut-on concevoir une liaison entre les deux phénomènes: changement de pigmentation du fruit et oxydation de la vitamine C que les exemples précédents nous ont montré en corrélation. Nous sommes réduits à des hypothèses, mais l'une des plus vraisemblables si l'on admet la théorie de Willstätter, est que l'acide ascorbique sert d'antioxydant au lycopène et aux lycopénoïdes qu'il protège de leur destruction. Il serait à lui, seul, ou pour partie, l'antioxydase entrevue par cet auteur.

b) Maturation de la pulpe d'une cerise de Montmorency: les fruits ont été cueillis sur une même branche au fur et à mesure des besoins analytiques: (dosages faits au dichlorophénol indophénol).

		En mg% de matière fraiche	
20 Mai	fruit vert (1 g 50)	Ac. ascorbique = 5	Ac. déhydro. = 3
9 Juin	fruit jaune (3 g 98)	,, 9	,, 7
,,	fruit rouge (4 g 06)	,, 10	,, 10

Il y a donc, comme chez les Solanées étudiées plus haut, augmentation constante de l'acide ascorbique et du quotient d'oxydation réversible qui devient égal à l'unité dans le fruit rouge. Le rougissement de la cérise a été particulièrement étudié par Obaton [26]; il a montré qu'il se produisait moins sous l'influence de la lumière que de la chaleur. Aussi avons nous fait l'essai suivant: nous avons prélevé sur un cerisier de Montmorency, trois branches aussi sem-

blables que possibles et semblablement exposées toutes trois portant une dizaine de fruits jaunes sur le point de mûrir, c'est à dire de tourner au rouge.

Nous avons dosé par le bleu de méthylène la vitamine C des fruits de la 1ère branche, ainsi que la matière sèche de la pulpe: c'est notre témoin de départ ou temoin No. 1.

La seconde branche a été mise à tremper par son extrémité inférieure dans un vase contenant de l'eau de source et laissée dans le laboratoire à la température de 16–18°. C'est notre témoin proprement dit ou témoin No. 2.

La troisième branche, trempant également dans l'eau de source, fut placée dans une étuve vitrée maintenue à la température de 35°. Au bout de 48 heures, les fruits avaient pris une teinte légèrement brunâtre mais n'avaient pas sensiblement rougi. Ils furent alors analysés avec ceux de la branche No. 2. Outre les deux formes ascorbiques et la matière sèche, nous avons dosé l'acidité totale et les sucres réducteurs par la liqueur de Fehling (mg % de tissus frais):

	Mat. sèche	Ac. asc.	Ac. déhyd.	Quot. d'ox. rév.	Ac. totale	Sucre réduct.
Branche No. 1	10,50	7,84	3,08	1/2,5	—	—
Branche No. 2	10,00	7,75	3,10	1/2,5	125 cc N/10	9,04
Branche No. 3	7,10	4,30	7,40	4/2,5	80 ,,	6,13

Comme pour le piment, le fait d'être coupé de la branche-mère et de tremper dans l'eau de source à la température de 16–18° n'a pas apporté de changement notable dans le taux de vitamine C des fruits de cette branche, puisque 1 et 2 sont identiques aux erreurs d'expérience près.

L'élevation de température subis par les fruits a eu pour corollaire une diminution importance de la matière sèche, résultant de la consommation des sucres et des acides organiques, ce qui est la conséquence normale d'une augmentation des échanges respiratoires. Ce sont là des phénomènes bien connus sur lesquels il est inutile de nous étendre: mais l'élément nouveau de ces déterminations, c'est la variation inverse des deux formes d'acide ascorbique et la brusque élévation du quotient d'oxydation réversible qui apparaît ici comme le traducteur fidèle de l'activité oxydo-réductrice de la vitamine C dans le fruit.

Si nous pouvons tirer de cet essai d'utiles renseignements sur la

corrélation existant entre les phénomènes respiratoires et l'acide ascorbique, nous ne pouvons pas en déduire la moindre conclusion quant à la liaison possible des variations de ce corps avec le rougissement des cerises.

c) Les agrumes ont fait l'objet d'un grand nombre de travaux. Nous avons pour notre part, étudié quelques pepins d'agrumes dont voici les teneurs obtenues par la méthode à l'indophénol. En mgr% de substance fraiche:

	As. ascor.	Ac. déhydro.	Total	Quot. d'ox. rév.
Citrons	44 mg	12 mg	56 mg	1/4
Oranges douces	22 ,,	22 ,,	44 ,,	1
Mandarines.	8 ,,	8 ,,	16 ,,	1
Pamplemousses. . . .	6 ,,	14 ,,	22 ,,	1,5

Les pépins de ces fruits qui en constituent la graine proprement dite sont donc encore très bien pourvus en vitamine C à l'époque de la pleine maturité du fruit; et non seulement celle-ci s'y trouve sous sa forme réduite, mais encore sous sa forme oxydée, dans des proportions telles que le quotient d'oxydation réversible est égal ou supérieur à l'unité.

§ 8. *Un fruit à graines amylacées.*

Sur *Phaseolus vulgaris*, var. Métis nain — nous avons étudié la formation de la gousse et du grain. La gousse très jeune, pesant à peine 4 g et dans laquelle les grains ne sont qu'à l'état embryonnaire dose (méthode à l'indophénol): Ac. ascorb. 15 mg% — ac. déhydro. 3 mg%. Huit jours plus tard, les grains sont toujours à peine développés: ils ne dépassent pas la grosseur d'une grosse tête d'épingle: la gousse pèse 7 g. Ac. ascorb. 22 mg% Ac. déhydro. 6 mg%.

Dans une gousse plus âgée, où les grains sont nettement formés, la part qui revient à chacun est la suivante:

Gousse 6 g 500 — Ac. ascorb. 71 mg% — Ac. déhydro. 17 mg%
Grains: 2 g 700 — Ac. ,, 100 mg% — Ac. ,, 0

Dans une gousse plus âgée, encore, et qui commence à jaunir, les chiffres deviennent:

Gousse: 5 g 500 — Ac. ascorb. 50 mg% — Ac. déhydro. 2 mgr%
Grains: 6 g 800 — Ac. ,, 20 ,, — Ac. ,, 0

La gousse passe donc par un maximum ascorbique (aussi bien

forme oxydée que forme réduite) au moment du plein développement du grain, alors que sa croissance propre atteint son maximum. Il nous manque, malheureusement le poids sec de chacun des constituants qui seul nous aurait permis de fixer avec exactitude le stade évolutif de chacun des échantillons.

§ 9. *Les fruits oléagineux.*

Cette sorte de fruits a retenu notre attention, à cause de la genèse des substances grasses qui se forment à partir des oses par réductions successives se résumant dans le schéma très simplifié suivant:

$$10\ C_6H_{12}O_6 \rightarrow \underset{\text{(trioleine)}}{C_{57}H_{104}O_6} + 3\ CO_2 + 8\ HO_2 + 20\ O_2$$

Il y avait donc là un domaine d'action possible pour l'acide ascorbique et c'est ce point particulier que nous avons tenté d'éclaircir.

Prenons le capitule d'*Hélianthus a.* en pleine floraison: des rangs du centre à ceux du pourtour, les formes ascorbiques varient comme suit dans les ovaires:

Centre: As. ascorb. 18 mg%, Ac. déhydro. 23 mg%
Milieu: „ „ 25 „ „ „ 18 „
Pourtour: „ „ 13 „ „ „ 15 „

Huit jours après la fin de la floraison des fleurs centrales les doses sont le suivantes:

	Pds frais.	% de mat. sèche	Ac. ascorb.	Ac. déhyd.	Q. d'ox. rév.
Le 26 Juillet					
Centre . . .	0,064 g.	16,77	7,2	5,6	3/4
Milieu . . .	0,128 g.	17,54	7,2	4,0	1/2
Pourtour . .	0,152 g.	20,30	6,8	3,2	1/2
Le 2 Août					
Pourtour . .	0,185 g.	20,80	2,7	16,0	6

Jusqu'à cette dernière date, il est impossible de séparer les téguments de l'amande qui n'est encore qu'un sac embryonnaire minuscule et excessivement fragile, mais le 16 Août on peut traiter à part les deux tissus; il n'y a pas trace de matière grasse dans l'amande.

	Pds frais.	% de mat. sèche	Ac. ascorb.	Ac. déhyd.	Q. d'ox. rév.
Le 6 Août					
Amande . . .	0,060 g.	23,0	1,8	0	0
Téguments . .	0,134 g	25,5	5,4	8,3	$\leqslant 1,5$

Le 12 Août les matières grasses apparaissent dans l'amande ; mais encore en très faible quantité : 0.12%.

	Pds frais.	%de mat. sèche	Ac. ascorb.	Ac. déhyd.	Q. d'ox. rév.
Le 12 Août					
Amande . . .	0,080 g	28	2,2	1,1	1/2
Téguments . .	0,100 g	40	5,6	2,8	1/2

Enfin le 18 Août la graine est presque mûre, le taux de matières grasses dans l'amande est important : 21%.

	Pds frais.	%de mat. sèche	Ac. ascorb.	Ac. déhyd.
Le 18 Août				
Amande	0,220 g	35	2,5	0,2
Téguments	0,080 g	50	0	0

Deux mois après la récolte dans une graine analysée au mois de Novembre, les chiffres sont les suivants :

	Pds frais.	%de mat. sèche	Ac. ascorb.	Ac. déhyd.
Le 13 Août				
Amande			2,5	1,1
Téguments			0	0

Au cours de l'évolution de cette graine, l'acide ascorbique qu'elle contient a bien été l'objet d'une oxydation très intense, mais celle-ci s'est produite un peu avant l'apparition des matières grasses dans l'amande, et s'est surtout maintenue ensuite dans les téguments. Donc, à moins que l'on ne démontre un jour que la lipogénèse à lieu dans les téguments, et que, de là, les matières grasses formées émigrent rapidement vers l'amande ; il est impossible de voir une liaison entre la lipogénèse et l'oxydation réversible ascorbique. Cette manière de voir est d'ailleurs confirmée par l'étude de l'olive et du pavot

Le 16 Juillet — très jeune capsule de pavot — les graines très molles et très tendres ne laissent aucune tache grasse sur le papier : (par le bleu de méthylène).

	Mat. sèche	Ac. ascorb.	Ac. déhyd.	Q. d'ox. rév.
Graines.	11,50%	34,4 mg%	12 mg%	1/3
Capsule	24,20%	58,8 mg%	18,6%	1/3

Le 26 Juillet — capsule un peu plus âgée — mais les graines ne laissent encore aucune tache grasse bien caractéristique sur le papier

	Mat. sèche	Ac. ascorb.	Ac. déhyd.	Quot. d'ox. rév.
Graines.	28,70%	34,4 mg%	8 mg%	1/4
Capsules.	42,50%	48,1 mg%	15 mg%	1/3

A cette même date une capsule plus avancée:

	Mat. sèche	Ac. ascorb.	Ac. déhyd.	Quot. d'ox. rév.
Graines.	34,40%	42,2 mg%	6,2 mg%	1/7

Le 5 Août — une capsule encore verte, mais en voie de déssèchement, les graines laissent, cette fois, une tache grasse bien caractéristique sur le papier:

	Mat. sèche	Ac. ascorb.	Ac. déhydr.	
Graines	53,0%	9 mg%	0 mg%	

Nous n'avons malheureusement pas eu le temps de faire des dosages des matières grasses des graines étudiées ci-dessus et nous avons dû nous contenter du test bien approximatif de la tache grasse sur le papier. Il est certain cependant que la lipogénèse débute très tôt, bien avant que la graine écrasée ne fasse une tache lipidique. Mais en admettant qu'elle soit déjà active à l'époque de nos premières analyses et que ce soit là une raison possible pour expliquer la valeur relativement élevée du quotient d'oxydation réversible, comment invoquer la même raison pour les parois de la capsule qui ont sensiblement même valeur pour leur quotient? Il est plus vraisemblable de penser qu'un même phénomène se déroulant à la fois dans les graines et dans les parois de la capsule, imprime une même valeur à leur quotient respectif.

Chez le péricarpe d'un fruit oléagineux comme l'olive dont la maturité a été ainsi décrite par Molliard [18]: „vers le 15 Août, alors que le noyau présente un poids de 170 à 400 mg la teneur en huile est de 4 à 15%; à la fin d'Août commence une 2ème période caractérisée par un accroissement rapide: elle se termine vers le début d'Octobre — la teneur en huile est alors de 30 à 62% dans

une 3ème période qui s'étend jusqu'au 15 Novembre, on observe une légère diminution du taux de l'huile qui peut s'abaisser à 50%".

10 Août: Ac. ascorb. 53 mg% Ac. déhydro. 8 mg% de substance fraiche

28 Sept.: Ac. ascorb. 30 mg% Ac. déhydro. 6 mgr% de substance fraiche

1er Décembre: olive commençant à noircir:

Ac. ascorb. 38 mg% Ac. déhydro. 4 mg% de substance fraiche

olive noire: Ac. ascorb. 16mg% Ac. déhydro. 0 mg% de substance fraiche

Il apparait donc pour tous les fruits ou graines oléagineux étudiés ci-dessus que:

1°) au fur et à mesure que les oses disparaissent pour faire place aux matières grasses, le taux d'acide ascorbique diminue.

2°) que le quotient d'oxydation réversible conserve une valeur sensiblement égale jusqu'à une phase avancée de la maturation et qu'il devient très faible ou nul à la maturité complète. Il est donc à rapprocher beaucoup plus des phénomènes respiratoires que de ceux de la lipogénèse.

Conclusions.

Lesf ruits sont des organes souvent assez riches en acide ascorbique; mais leur teneur va en diminuant de la périphérie au centre du fruit (abords du noyau).

Elle augmente en valeur absolue et relative dans un fruit du début à la fin de la formation de celui-ci.

Les recherches que nous avons entreprises ont mis en évidence une certaine corrélation entre les variations du quotient d'oxydation réversible et certains changements de pigmentation des fruits qui approchent de leur maturite.

Elles n'ont pas réussi, par contre à faire apparaitre la moindre corrélation entre ce quotient et la lipogénèse; si celle-ci a bien lieu „in situ" dans la graine et non dans les téguments de la graine ou du fruit comme il apparait logique de le penser.

Le quotient d'oxydation réversible semble donner une idée plus exacte du rôle respiratoire de l'acide ascorbique que le seul dosage de la forme réduite et la constation d'un stock plus ou moins important de cette forme dans le fruit.

Chapitre IV

Influence des conditions extérieures sur la teneur des végétaux en vitamine C

Nous distinguerons les conditions de nutrition de la plante et les conditions écologiques ou climatériques.

§ 9. *Nutrition de la plante.*

Influence des substances minérales: Bien que n'ayant apporté aucune contribution personnelle à cette question qui à déjà fait l'objet de multiples recherches, nous avons fait l'exposé bibliographique rapide de ce chapitre qui apporte des données importantes à la conception du rôle général de l'acide ascorbique.

a) Influence des sels azotés (nitrates ou sels d'ammonium).

L'azote est un des éléments principaux de la nutrition minérale de la plante, généralement apporté aux végétaux cultivées par les engrains azotés; aussi est-ce un des éléments minéraux qui a été le plus étudié par un grand nombre de chercheurs.

En dernier lieu: Borje Aberg et Ivar Ekdahl[1]) trouvent que l'acide ascorbique pour 100 de matière fraiche passe par un minimum avec les faibles fumures azotées; mais ils notent une certaine corrélation positive entre la teneur en acide ascorbique et la matière sèche des feuilles

Sur les tomates les essais des deux auteurs précités montrent un accroissement de l'acide réduit dans les feuilles des plantes jeunes sous l'influence de la fumure azotée, et une réduction qui peut aller du double au simple chez les feuilles des plantes plus agées cultivées en serre, d'Octobre à Février. En été, ce sont les doses les plus élevées qui provoquent la plus grande concentration en acide ascorbique dans les feuilles et les fruits. Il apparait alors qu'une même dose d'azote, nuisible à la plante lorsque l'éclairement est insuffisant, lui devient profitable lorsque celui-ci redevient normal pour la plante. Dans le 1er cas, cette dose provoque une baisse de l'acide ascorbique; dans le 2ème cas, une élévation. La concentration ascorbique dans les tissus serait en rapport avec la vigueur et l'équilibre végétatif de la tomate. B. Aberg et I. Ekdall s'appuyant sur les remarques précédentes, émettent l'hypothèse que l'acide ascorbique est un facteur de croissance.

L'action de l'azote ammoniacal a été étudiée en détail par L. W. Mapson et Z. M. Cruickshand[15]) sur les jeunes plantes de cresson: avec les plus hautes concentrations (0,1 m) l'effet dépressif du sulfate d'ammoniaque fut considérable et se marquat aussi bien sur la croissance de la plantule que sur le taux relatif d'acide ascorbique dans les tissus frais. Avec des doses plus reduites (0,02 m) la croissance fut normale mais le taux de vitamine C fut néanmoins très réduit. Il n'y a donc pas de correlation posi-

tive entre la réduction de la croissance et la réduction du taux ascorbique.

Si l'on en juge par le tableau ci-dessous donné par Mapson et Cruickshand, l'acide déhydroascorbique est moins influencé que la forme réduite par l'azote ammoniacal.

Solution nutritive	Durée de l'expérience en heures	Acide ascor.		Acide déhydro.		Acide total	
		Concent. en mg/g	Total produit par 100 feuilles	Concent. en mg/g	Total produit par 100 feuilles	Concent. en mg/g	Total produit par 100 feuilles
Eau distil . . .	90	0,485	0,86	0,08	0,14	0,55	1,0
E.D. + 0,02 m .	90	0,215	0,35	0,085	0,13	0,30	0,48
Sol. nutrit. . . .	90	0,365	0,77	0,051	0,105	0,47	0,87
S.N. + 0,02 m .	90	0,145	0,28	0,06	0,11	0,205	0,39

Mais l'effet dépressif du sel d'ammonium employé ci-dessus, semble dû à l'anion SO_4 beaucoup plus qu'au cation NH_4 puisqu'il ne se produit pas avec les sels d'ammonium des acides: carbonique nitrique, acétique ou succinique.

Les sels de sodium et de potassium des quatre acides ci-dessus augmentent la quantité d'acide ascorbique produit restant dans les tissus, cependant que les acides eux-mêmes sont sans action sur ce phénomène. Ces sels ont une action antagoniste du sulfate d'ammonium — mais non leur acide.

Les deux auteurs ont noté une corrélation positive entre la valeur du pH du suc cellulaire et le total d'acide ascorbique synthétisé. Enfin, ils attribuent l'action des sels sur le taux de synthèse de l'acide ascorbique, à la quantité de base alcaline restant libre pour la plante après les échanges du métabolisme. Cette dernière hypothèse est évidemment très discutable, les auteurs n'ayant pas vérifié si les variations trouvées dans les tissus frais résultent d'une synthèse moins grande ou d'une disparition plus rapide: c'est là d'ailleurs, une critique générale que l'on peut faire à toutes les études sur la synthèse ascorbique dans l'impuissance où nous sommes de mesurer la quantité d'acide ascorbique qui disparait chaque jour d'un végétal entier ou d'un tissu déterminé. L'étude du quotient d'oxydation réversible de cette plante ou de ce tissu peut cependant nous donner de précieuses indications sur cette disparition: il faut voir en lui, moins un critérium absolu qu'un fil conducteur propre à nous permettre de débrouiller un écheveau passablement compliqué. Or si nous nous reportons au tableau ci-dessus, nous voyons que l'addition de 0,02 m de sulfate d'ammonium soit à l'eau distillée, soit à la solution nutritive a pour effet d'augmenter considérablement le quotient d'oxydation réversible qui dans le premier cas passe de 1/6 à plus de 1/3 et dans le second, de 1/7 à près de 1/2, ce qui tendrait à indiquer que la diminution d'acide réduit constaté par les auteurs est le fait d'une oxydation plus grande et non de la réduction de la synthèse. A priori, il était d'ailleurs surprenant qu'une plantule vivant sur eau distillée pure puisse synthétiser plus d'acide ascorbique qu'une plantule vivant sur une solution nutritive appropriée.

Cette étude de Mapson et Cruickshand a eu le mérite de mettre en lumière l'action réciproque des éléments nutritifs entre eux et des facteurs écologiques sur lesquels nous reviendrons plus loin.

b) Influence du potassium, du calcium, du magnésium, des anions sulfurique et phosphorique, et des oligoéléments.

Une étude tres complète de ces facteurs nutritifs a été faite par Stanley, A. Watson et G. R. Noggle [26]) sur les jeunes plants d'avoine. Ils ont trouvé que la suppression du potassium et surtout du magnésium dans la solution nutritive avait pour effet d'augmenter considérablement le taux d'acide ascorbique des feuilles. La suppression des sulfates et phosphates augmente très légèrement ce même taux, tandis que la suppression du calcium et des nitrates le réduisent un peu pour le calcium, beaucoup pour les nitrates.

Les phénomènes sont les mêmes dans la tige, sauf en ce qui concerne le potassium, dont la disparition dans la solution nutritive provoque une légère diminution.

Devant l'augmentation considérable de l'acide réduit en l'absence de magnésium, les auteurs hésitent entre les deux hypothéses possibles: synthèse plus grande ou utilisation moindre. Ils penchent d'ailleurs pour la première hypothèse, qui nous parait cependant la moins vraisemblable; en effet, étant donné le rôle du magnésium et du potassium dans la synthèse, et la polymérisation des oses, il serait surprenant que leur disparition provoque la formation surabondante d'un corps aussi voisin des oses que la vitamine C — tandis que la non utilisation de celui-ci apparaît comme la conséquence logique de l'interruption de la chaîne métabolique provoquée par l'absence des deux métaux alcalins ou alcalino-terreux. Il y a même une indication supplémentaire dans le fait que l'absence de potassium provoque une augmentation dans les feuilles et une diminution dans les tiges, cette observation met en lumière non seulement l'opposition des deux métaux avec les autres ions nutritifs, mais leur opposition entre eux et l'antagonisme des feuilles et des tiges dans la métabolisme ascorbique: celui-ci ne doit pas être simple, mais doit émarger à un certain nombre de systèmes, probablement à un ou plusieurs systèmes anaboliques d'une part et à un ou plusieurs systèmes cataboliques d'autre part.

Feres et Brown [7]) ont étudié l'influence des oligoéléments sur la richesse en vitamine C des légumes à feuilles ou à tubercules. Ils ont trouvé que le zinc seul avait une influence positive (accroissement du taux d'acide ascorbique). Mais Brown, Patton, Blythe et Shetland [4]) notent une augmentation pour les cultures de bettes (*Beta vulgaris*) dépourvues de manganèse, magnésium, potassium et azote.

Enfin Chandler et Miller [6]) cultivant des rutabagas, notent une élévation du taux d'acide ascorbique dans les cultures ayant reçu du bore. Mais bien des auteurs, parmi lesquels Amner et Nightingale, ont

noté que l'influence des éléments nutritifs minéraux, était moindre que celle des facteurs écologiques entourant les cultures et que, parmi ceux-ci, l'éclairement et la température ont une influence prépondérante sur la richesse des tissus en acide ascorbique.

§ 10. *Influence de la lumière et de la température.*

Mapson et Cruickshand, dans leur étude sur les jeunes plants de cresson ont montré que l'action dépressive du sulfate d'ammonium se manifeste aussi bien à l'obscurité qu'à la lumière, malgré la réduction de 50% du taux d'acide ascorbique observé chez les plantules venues à l'obscurité.

D'autres observations ont été faites sur ce sujet par divers auteurs: entre autres Mac Intosh[14]) remarque que le teneur des plantes en acide ascorbique s'accroit par exposition au soleil.

Giroud[9]) puis Moldtmann[10]), Reid[22]), Smith et Gillies[24]) avaient signalé une certaine fluctuation diurne du taux d'acide ascorbique, celui-ci ayant son maximum au milieu de la journée.

Mais il faut attendre Börje Aberg pour avoir une étude systématique des deux principales influences qui s'exercent sur la synthèse ascorbique: la température et l'éclairement avec son intensité, sa durée journalière ou photo période et la qualité de sa lumière. Les conclusions de cet auteur sont les suivantes:

1°) La lumière bleue — et, d'une manière générale, les radiations lumineuses comprises entre 400 et 500 μ sont sans effet sur le taux des feuilles en acide ascorbique. Les radiations ultra-violettes n'ont pas d'effet ou un léger effet dépressif.

2°) La quantité d'acide ascorbique contenue dans une feuille diminue en même temps que l'intensité lumineuse chez les jeunes tomates. Mais des plantes conservées longtemps à l'obscurité, contiennent encore une dose très notable d'acide ascorbique. Il y a donc plusieurs modes de synthèse ascorbique dans la plante et la lumière n'est pas indispensable à l'un d'eux. De plus, à l'obscurité ce sont les feuilles No. 3 et 4 (en partant du sommet de la tige) qui perdent le plus d'acide ascorbique. Il y a donc migration de la vitamine C à partir de ces feuilles et des feuilles plus agées, vers les feuilles les plus jeunes.

3°) Chez la tomate, la quantité d'acide ascorbique contenue dans les feuilles, augmente chez les plus agées avec l'allongement de la photopériode, mais chez les plus jeunes, il y a un plafond à partir de 12 heures d'éclairement journalier. Ce fait s'explique par les troubles de croissance provoqués chez la tomate par un éclairement journalier trop long (18 et 24 heures) — troubles qui suspendraient la migration des produits élaborés et en particulier de l'acide ascorbique, des feuilles agées vers les plus jeunes.

4°) L'augmentation de la temperature de 15 à 23° a provoqué chez de jeunes plants de tomates une diminution d'environ 30% de la quantité d'acide réduit contenu dans les feuilles. L'auteur pense que ce fait est en

rapport avec l'augmentation de la vitesse de croissance de la plante. La synthèse ascorbique est peut-être plus active à 23° qu'à 15°, mais son catabolisme est activé dans une proportion supérieure.

§ 11. *Observations personnelles sur l'influence de la lumière et de la température.*

Des nombreux dosages que nous avons effectués sur *Ribes nigrum* nous avons tenté d'extraire quelques indications relatives à l'influence de la lumière et de la température sur la richesse en acides ascorbique et déhydroascorbique des feuilles de cet arbrisseau. Malheureusement, nos sujets étant situés en pleine terre et à l'air libre, étaient sousmis aux conditions météorologiques, c'est-à-dire à un ensemble de conditions dont nous pouvions bien enregistrer certaines données, mais non pas les modifier. Il en est résulté que la plupart de nos observations sur ce sujet n'ont pas toute la rigueur et la précision désirables, que certains éléments nous échappent, n'ayant pu, suivant la pensée de Descartes, „diviser le problème en autant de parties qu'il est nécessaire pour les mieux résoudre".

D'autre part, l'Office National Météorologique qui a aimablement mis à notre disposition le relevé journalier des températures maxima et minima, et la somme des heures d'insolation, n'a pu nous fournir d'indications sur l'intensité lumineuse journalière qui eut été cependant une donnée importante dans la résolution du problème que nous proposons actuellement.

Voici les dosages comparatifs faits sur les feuilles de *Ribes nigrum*, mis en regard: 1°) des sommes de températures maxima et minima et d'insolation du jour du dosage et des 48 heures qui l'ont précédé. — 2°) des températures maxima et minima et du total d'heures d'insolation de la seule journée du dosage. Ces chiffres sont ceux relevés à l'observatoire du Parc St Maur à Paris.

Le prélèvement des échantillons a toujours été fait entre 13 et 15 heures. — Les flèches indiquent le sens de la variation entre la 1ère et la 2ème observation.

Les feuilles sont numérotées en partant de l'extrémité supérieure du rameau, dont le bourgeon terminal ou la très jeune feuille a peine sortie de lui, portent le No. zéro.

Date du prélèvement.	No. de la feuille	En mg% de tissus frais						1e pour les 3 jours				2e pour la journée			
		Ac. asc.		Ac. déhyd.		Q. d'ox. rév.		Σ des températures maxim. minim.		Σ des heures d'insolation.		Σ des températures maxim. minim.		Σ des heures d'insolation.	
19 Avr. 1947	3ème	210	↘	20	↗	1,10	↗	67,9 + 28,2	↘	23,1	↘	20,3 + 9,3	=	8,3	↗
3 Mai 1947	3ème	185	↘	80	↗	4/9	↗	50,6 +19,8	↘	19,4	↘	20,8 + 8.7	=	9,2	↗
19 Avr. 1947	4ème	176	↗	11	=	1/17	↗	67,9 +28,2	↗	23,1	↗	20,3 + 9,3	=	8,3	↗
3 Mai 1947	4ème	194	↗	10	-	1/19	↗	50,6 +19,8	↗	19,4	↗	20,8 + 8,7	=	9,2	↗
20 Juin 1947	1ère	134	↘	14	↗	1,10	↗	73,7 + 43,2	↘	16,7	↗	21,7 + 14,8	↗	1,7	↗
24 Juin 1947	1ère	73	↘	27	↗	1/3	↗	71,7 +34,3	↘	34,8	↗	28,9 +12,0	↗	14,9	↗
20 Juin 1947	3ème	123	↘	0	↗	0	↗	73,7 + 43,2	↘	16,7	↗	21,7 +14,8	↗	1,7	↗
24 Juin 1947	3ème	98	↘	28	↗	1/4	↗	71,7 +34,3	↗	34,8	↗	28,9 +12,0	↗	14,9	↗
22 Juin 1947	1ère	100	↗	18	↗	1,5,5	-	62,8 + 42,3	↗	11,0	↗	20,3 + 12,2	↗	6,0	↗
26 Juin 1947	1ère	162	↗	27	↗	1/6	-	105,0 +44,3	↗	42,1	↗	36,5 +16,1	↗	12,4	↗
19 Sept. 1947	4ème	154	↘	4	↘	1/40	↗	90,6 + 49,5	↗	22,5	↘	32,9 +17,0	↘	7,3	↗
26 Sept. 1947	4ème	150	↘	0	↘	0	↗	50,3 +24,2	↗	12,9	↘	16,9 +12,1	↘	3,2	↗
3 Avr. 1948	4ème	156	↗	30	↘	1/3	↗	37,8 + 15,9	↗	17,3	=	12,4 + 4,6	↗	9,2	↗
17 Avr. 1948	4ème	175	↗	8	↘	1/22	↗	50,3 +17,3	↗	17,7	=	19,0 + 7,3	↗	1,7	↗
24 Avr. 1948	1ère	227	↘	63	↗	1,4	↗	55,9 + 26,1	↗	14,1	↗	18,5 + 7,7	↘	4,1	↗
1er Mai 1948	1ère	137	↘	87	↗	3/5	↗	45,9 +20,2	↘	27,1	↗	15,9 + 6,1	↘	4,7	↗
24 Avr. 1948	4ème	219	↘	27	↗	1/8	↗	55,9 + 26,1	↘	14,1	↗	18,5 + 7,7	↘	4,1	↗
1er Mai 1948	4ème	149	↘	81	↗	1/2	↗	45,9 − 20,2	↘	27,1	↗	15,9 + 6,1	↘	4,7	↗
8 Mai 1948	3ème	196	↗	58	=	2/7	↗	66,0 +30,5	=	31,2	↗	25,0 10,6	↘	13,3	=
22 Mai 1948	3ème	216		56	-	1/4	↗	64,5 + 30,5	=	36,9		21,2 + 9,3	↘	13,7	=
22 Mai 1948	1ère	219	↘	20	↘	1/11	↗	64,5 +30,5	↗	36,9	↗	21,2 + 9,3	↘	13,7	↗
29 Mai 1948	1ère	196	↘	12	↘	1/16	↗	52,1 +23,2	↗	14,6	↗	15,5 + 9,0	↘	3,0	↗
16 Sept. 1948	4ème	174	↗	10	↗	1/16	↗	58,5 +35,0	↗	9,2	↗	18,6 +10,6	↗	0,6	↗
27 Sept. 1948	4ème	194	↗	21	↗	1/10	↗	78,0 +30,6	↗	29,3	↗	28,3 +14,0	↗	9,2	↗

Les indications qui se dégagent de ce tableau sous les réserves que nous avons indiquées plus haut et en remarquant, en outre, que les phénomènes de floraison viennent interférés sur les observations faites dans la 2ème quinzaine d'Avril, sont les suivantes:

chez les feuilles supérieures d'un rameau de *Ribes nigrum*, les variations de l'acide ascorbique sont généralement de même sens que les variations de la température,

tandis que les variations de l'acide déhydroascorbique sont généralement de même sens que les variations de la durée d'insolation.

Mais en ce qui concerne les variations de la forme réduite, il y a parfois antagonisme entre la température et l'éclairement: la température, ayant pour effet d'accroître le catabolisme de ce corps, en accélére la disparition, tandis que l'éclairement, qui favorise son anabolisme, favorise son accumulation dans les feuilles. Il en résulte des indications discordantes, suivant que l'un ou l'autre de ces phénomènes a une action prépondérante.

Les variations de la forme oxydée, dont la concordance avec les variations d'insolation est bien meilleure qu'avec les variations de température, apportent un commencément de preuve en faveur de l'opinion d'un certain nombre d'auteurs, parmi lesquels Giroud, Ratsimamanga et Leblond, Mentzer, French, West et Ney. Ces biologistes ont pensé que l'acide ascorbique participait au processus même de la photosynthèse, c'est à dire à la synthèse des glucides et Mentzer [17]) à remarqué à ce propos, que l'oxydabilité, considérablement accrue par la lumière, de l'acide ascorbique, serait un point avantageux pour ce processus.

D'autre part, French [8]) a isolé par vibrations ultrasonores les pigments assimilateurs de certaines bactéries et a vu qu'ils accéléraient considérablement l'oxydation de l'acide ascorbique en présence de lumière. Le phénomène varie avec les longueurs d'onde et l'intensité du flux lumineux.

De leur côté, West et Ney [28]) ont montré que la présence d'acide ascorbique en milieu alcalin, accélérait considérablement la polymérisation des aldéhydes dont l'aldéhyde formique en oses.

Temps d'observation	Substances réductrices formées à partir de	
	H.C.HO + Ca(OH) sans acide ascorbique	H.CHO + Ca(OH) avec acide ascorbique
0 minute	0	24
60 ,,	0	391
120	11	269
180 ,,	154	199

Rapprochons ces observations de celles que nous avons précédemment rencontrées, à savoir:

L'influence de la nutrition azotée qui dans la mesure où elle favorise la formation de la chlorophylle, favorise également celle de l'acide ascorbique.

L'influence de deux carences minérales: la magnésienne qui entrave la formation de la chlorophylle, la potassique, qui trouble gravement le métabolisme glucidique en empêchant la polymérisation des oses, favorisent toutes deux une accumulation insolite d'acide réduit, comme si la diminution de l'assimiliation chlorophyllienne la laissait sans emploi.

Les deux mécanismes d'apparition de l'acide ascorbique, à la lumière et à l'obscurité comme si l'assimilation chlorophyllienne et la respiration engendraient chacune l'acide ascorbique dont elles avaient besoin.

L'influence prépondérante de la lumière dans la formation de l'acide déhydroascorbique.

L'influence dualiste de la température qui augmente et la synthèse et la destruction de l'acide ascorbique, pourvu que la plante soit exposée à la lumière, sans quoi la destruction est seule perceptible, ainsi que nous allons le monter au paragraphe suivant.

Il y a là tout un ensemble de faits qui militent singulièrement en faveur d'un rôle actif du système ascorbique dans le mécanisme de la photosynthèse.

De quelle nature peut être cette intervention? C'est encore sous forme de transporteur ou d'activeur d'hydrogène que le rôle de l'acide ascorbique nous semble le plus vraisemblable. Cette notion de transmetteur d'hydrogène qui n'est plus discutée dans la dégradation des corps, nous semble également devoir s'imposer dans leur synthèse — d'une part à cause du parallélisme rigoureux des deux ordres de phénomènes: anabolisme et catabolisme — et d'autre part parcequ'il serait bien étonnant que l'hydrogène provenant de la photolyse de l'eau, puisse se fixer directement sur le gaz carbonique, s'il n'y a pas entre eux au moins un de ces activateurs qui permettent ce genre d'union dans le catabolisme. On voit mal, d'autre part, l'oxygène moléculaire libéré au sein des chloroplastes où il ne manquerait pas de causer des oxydations dangereuses pour le protoplasme. On conçoit très bien par contre, l'acide ascorbique intervenant à ce moment là pour transformer en péroxyde d'hydrogène,

l'oxygène libéré par la photosynthèse. Cette hypothèse rend compte, à notre avis du fait que nous avons constaté de la richesse relative en acide déhydroascorbique des tissus conducteurs qui sont la voie d'évacuation normale des produits de la photosynthèse.

Pour en revenir à l'influence de la lumière et de la température sur le taux de vitamine C dans le système foliaire, nous avons tenté de résoudre la question en séparant ces deux facteurs dans l'expérience suivante:

Six pots aussi semblables que possible et remplis de sable de rivière bien homogénéisé, ont été ensemencés le 17 Avril avec des petits pois de la variété Sénateur. Trois plantes ont été laissées dans chaque pot, et le 29 Mai, alors que les plantes comptaient six étages de foliles — deux pots désignés par A ont été laissés en place dehors, comme témoins —

deux autres pots B ont été transportés au laboratoire, à une température moyenne de 20° et placés à l'obscurité complète:

les deux derniers pots C ont été mis dans la glacière à une température de +1 à 2°, à une obscurité complète;

48 heures après les 18 plantes ont été récoltées et analysées; les résultats ont été les suivants:

		En mg% de tissus frais		
		Ac. ascorb.	Ac. déhyd.	Quot. d'O. R.
Pots A	Stipules et folioles	113	4	1/28
	Tiges	42	12	3/11
Pots B	Stipules et folioles	47	2	1/24
	Tiges	20	6	3/10
Pots C	Stipules et folioles	112	4	1/28
	Tiges	42	11	3/11

Un accident nous à malheureusement privé du poids sec de ces plantes, mais leur substrat sableux ayant été maintenu à son maximum d'humidité, nous pouvons, sans erreur appréciable estimer qu'il était le même dans les trois sortes de pots.

Du 27 au 29 Mai, les conditions météorologiques auxquelles sont restés soumis les pots A ont été les suivantes: température moyenne maxima: 17,9, température moyenne minima: 7,7. Somme des heures d'insolation: 14,6.

La comparaison des pots A et B fait ressortir que l'absence complète de lumière, jointe à une légère augmentation de température à provoqué une diminition de plus de 50% du taux d'acide ascorbique

oxydé et réduit, aussi bien dans les stipules et foliles que dans les tiges.

La comparaison des pots A et C fait ressortir qu'une température suffisamment basse pour ralentir la plupart des réactions métaboliques de la plante (sans nuire toutefois à la vitalité de celle-ci) assure la conservation des deux formes ascorbiques, même en l'absence totale de lumière.

La comparaison des pots B et C montre qu'une élévation de température, non compensée par un éclairement suffisant, diminue rapidement le taux des deux formes ascorbiques dans la plante (tissus conducteurs et tissus assimilateurs).

L'action opposées de la lumière et de la température sur le taux des tissus foliaires en acide déhydroascorbique qui nous avait échappé par l'observation de *Ribes nigrum* à l'air libre, existe donc, au moins chez *Pisum sativum*.

§ 12. *Influence des solutions sucrées comme source alimentaire des feuilles détachées de leur tige.*

Nous avons fait l'essai suivant: dans une planche de haricots Noir de Belgique, au moment de la floraison, au mois de Septembre, par journée ensoleillée, nous avons à 8 heures du matin, cueilli sur quelques pieds, deux feuilles déterminées que nous avons réparties en deux groupes: les pétioles du premier trempaient dans l'eau distillée, tandis que les pétioles du second trempaient dans une solution de dextrose à 3%. Les deux groupes furent laissés au milieu de la planche, auprès de leurs pieds-mères et exposés comme eux. A 17 heures, des feuilles semblables aux précédentes furent cueillies sur les mêmes pieds et les deux formes ascorbiques dosées dans les trois groupes de feuilles:

	Ac. ascorb.	*Ac. déhydro.*	
1°) feuilles restées sur pieds	110 mg%	59 mg%	tissus
2°) feuilles sur eau distillée	130 ,,	70 ,,	frais.
3°) feuilles sur sol. à 3% de dext. . .	155 ,,	60 ,,	,,

Nous n'avons malheureusement pas pu calculer la matière sèche de ces trois groupes de feuilles, mais le moins turgescent et le moins aqueux ne pouvait être que celui des feuilles témoins, restées sur pied, ce qui augmenterait encore la différence en faveur des deux autres groupes. Si l'on considère l'eau distillée comme équivalente à la sève brute pendant la durée de l'expérience (soit de 8 à 17 heures),

il semble possible d'évaleur la quantité de vitamine C élaborée par une feuille de haricot Noir de Belgique, pendant cette même durée et qui serait de: acide ascorb. 130—110 = 20 mg%
acide déhydro. 70—59 = 11 mg%
de tissus frais, soit environ le 1/5 des quantités totales (feuilles jeunes, en pleine activité fonctionnelle, mais totalement développée et par journée ensoleillée).

Les oses augmentent considérablement le taux de l'acide réduit dans les feuilles, mais semblent sans, effet ou d'un effet inhibiteur, sur la formation d'acide oxydé. Si l'on considère que les jeunes feuilles d'un rameau reçoivent des plus âgées non seulement de l'acide ascorbique, mais encore les oses de la sève élaborée, on aura là l'explication: de la richesse de certaines jeunes feuilles en acide réduit — et de la pauvreté ou de l'irrégularité de la forme oxydée dans ces mêmes feuilles.

Conclusions.

Les actions fertilisantes, dans la mesure où elles sont profitables à la plante et augmentent sa croissance tout en respectant son équilibre végétatif, augmentent aussi la quantité d'acide ascorbique réduit, contenu dans les tissus. Toutefois, les carences magnésienne et potassique qui provoquent des troubles graves dans la photosynthèse et le métabolisme des glucides, et par là dans la physiologie toute entière de la plante, provoquent une augmentation du taux d'acide réduit chez les feuilles.

La lumière augmente le taux d'acide ascorbique dans les feuilles, par augmentation de sa synthèse; toutefois, une partie de l'acide ascorbique prend naissance à l'obscurité. L'élévation de la température jusqu'à un maximum non déterminé, doit également favoriser la synthèse ascorbiques; mais par une action antagoniste, elle favorise aussi son catabolisme et sa destruction, de sorte que, l'action résultante est souvent une diminution du taux d'acide ascorbique au sein des tissus.

L'accumulation d'acide déhydroascorbique dans les feuilles, est sous la dépendance de l'éclairement, sans qu'il nous ait été possible de faire la part revenant à chacun des deux facteurs: intensité et durée d'éclairement. Divers faits, dont celui-ci, militent activement en faveur de l'hypothèse d'une intervention du système ascorbique dans le processus de la photosynthèse.

RÉSUMÉ ET CONCLUSION

Au cours de cette étude, nous avons cherché à préciser les variations chez les végétaux supérieurs des deux formes de l'acide ascorbique, de leur rapport mutuel et de leur équilibre que nous avons appelé le quotient d'oxydation réversible de l'acide ascorbique.

L'étude de ce rapport nous semble une étape indispensable à la bonne compréhension du véritable rôle d'un corps dont la principale propriété connue est une oxydabilité réversible tout à fait remarquable et qui doit agir tantôt comme intermédiaire d'oxydation, tantôt comme intermédiaire de réduction.

Nous avons suivi ces variations dans les feuilles, les inflorescences et les fruits de certaines plantes, et cherché à préciser le rôle des influences extérieures, particulièrement des facteurs lumière et température sur ces variations.

Les variations du quotient d'oxydation réversible nous ont permis de confirmer l'opinion des auteurs qui ont assigné au système ascorbique l'un ou l'autre, ou plusieurs à la fois des rôles suivants:

— tampon d'oxydo-réduction lors d'une oxydation ou d'une réduction très active au sein des tissus et qui pourrait être dangereuse pour eux,

— transporteur d'hydrogène dans certains des phénomènes ordinaires de la respiration,

— transporteur d'hydrogène au cours des phénomènes de l'assimilation chlorophyllienne.

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